UNIVERSITY OF ALBERTA

THE EVALUATION OF VASOACTIVE INTESTINAL POLYPEPTIDE AND ENVIRONMENTAL TEMPERATURE ON INTAKE, DIGESTIBILITY, METABOLISM AND GASTROINTESTINAL MOTILITY IN SHEEP

BY

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DEDICATION

TO MY PARENTS FOR THEIR LOVE AND EDUCATION

TO MY WIFE, SHENGWEN ZHAO,
SON, MINGYU LI, AND DAUGHTER, MINGZHI LI
FOR THEIR LOVE, PATIENCE AND SUPPORT

ABSTRACT

A total of five experiments were conducted to test the hypothesis that vasoactive intestinal polypeptide (VIP) will increase feed intake, gastrointestinal electromyographic (EMG) activity, nutrient digestion in forestomachs and metabolic rate in sheep under different environmental temperatures (1 ± 2 vs 21 ± 2 °C) using crossover experimental designs in warm and cold environmental temperatures. Alfalfa pellet diets were offered, *ad libitum*, for 8 h per day or 24 h per day to fistulated sheep. VIP (1-1.5 nmol/min) or saline (control) was infused through a gastric-artery catheter intermittently for 10 min periods every 30-60 min for 3 h each day. Vasoactive intestinal polypeptide inhibited ($P < 0.05$) EMG activities of the reticulum, reticulo-omasal orifice (ROO) and omasum starting about 3 min after initiation of infusion. The inhibition was transient and start to return to pre-infusion values at about 8 min during VIP infusion and no inhibition lasted beyond 1.5 min after stopping infusion of VIP. The duration of the ROO opening after the second reticular contraction was increased by about 2 seconds by VIP infusion ($P < 0.05$). In sheep on an 8 h feeding schedule, VIP treatment increased ($P < 0.05$) feed intake by 14.3-26.1% (1.18 vs 0.98 kg/d), compared to saline infusion. However, duodenal passage of dry matter (DM), crude protein (CP), bacterial N, and amino acids (AA) were not influenced ($P = 0.14$-0.40) by VIP. In sheep on a 24 h feed schedule, there was no effect of VIP on feed intake (2.12 vs 2.08 kg/d), duodenal passage of DM, CP, bacterial N, and AA under both warm and cold environments. Vasoactive intestinal polypeptide infusion increased ($P < 0.05$) heart rate, but the metabolic rate of the sheep was not influenced during periods of VIP infusion. Cold exposure of sheep resulted in significant ($P < 0.05$) increases in feed intake (2.27 vs 1.77

kg/d), and duodenal digesta passage of DM, CP, AA and bacterial N, compared to that in the warm environment. Cold exposure of sheep reduced ($P < 0.05$) the active duration of EMG cycle activities of the reticulum, the quiescent time of the ROO EMG and both the active time and quiescent time of the omasal EMG cycles. The frequency of omasal EMG cycles was increased as well when animals were exposed to a cold environment. The results of these experiments indicate that VIP exerts effects on feed intake of sheep, likely by its action on smooth muscle of the gastrointestinal tract.

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Experiment	Chapter two	Chapter three	Chapter four	Chapter five	Chapter six
1	*	*		*	
2	*		*	*	
3	*		*	*	
4			*	*	
5	*	*			
6					*

CHAPTER ONE

GENERAL INTRODUCTION

Studies were carried out with sheep to determine the effect of VIP and environmental temperatures on intake, digestion, metabolism and gastrointestinal motility in sheep fed a diet of alfalfa pellets. For this reason, the first part of the literature review is presented on the physiological role of VIP, with emphasis on regulation of gastrointestinal motility and heart rate, and on potential evidence for improvement of intake and digestion. The second part of the review focuses on the effect of environmental temperatures on intake, digestion and metabolic rate. Further, studies were carried out with sheep on metabolic effects of water restriction. Water is fundamentally important for nutrient digestion and other physiological processes such as transport of metabolites. Therefore, the third part of the review focused on water restriction, with particular reference to the regulation of metabolic rate and intake in sheep.

LITERATURE REVIEW

A. Biology of vasoactive intestinal polypeptide

Vasoactive intestinal polypeptide (VIP) is a highly basic 28 amino acid peptide with a molecular weight of 2236 (Rayford et al., 1976) and which was first isolated from porcine small intestine (Said & Mutt, 1970). VIP belongs to a rapidly expanding family of cerebrogastrointestinal oligopeptides (Hernandez et al., 1987). There are number of similarities in structure to secretin, gastric inhibitory polypeptide (Table 1-1), and glucagon, and also some sequences similar to those of growth hormone releasing hormone (Itoh et al., 1983; Prysor-Jones and Jenkins, 1988). Mammalian VIP is identical in pig, cow, human, rat, dog and goat but differs in the guinea pig (GP) in positions 5, 9, 19, and 26 (Raufman et al., 1986). The gene encoding the preprovasoactive intestinal polypeptide precursor molecule and the VIP receptor have been identified. The mRNA for VIP has also been identified and

quantified by molecular biological methods. The biological actions of VIP have been elucidated by both *in vivo* and *in vitro* methods (Ottesen and Fahrenkrug, 1995).

Distribution

Vasoactive intestinal polypeptide fulfils many of the classical criteria for neurotransmission and has a broad spectrum of biologic actions with its wide-spread neuronal localization in the central and peripheral nervous system (Molinero et al., 1985; Luis et al., 1988; Sidawy et al., 1989; Wakade et al., 1991; Fahrenkrug, 1993; Chakder and Rattan, 1995; Ottesen and Fahrenkrug, 1995). VIP delivery to its sites of activity may be from the circulation (eventually released by nontarget cells) or is secreted locally within the target tissue (Blank et al., 1988). VIP-ergic neurons are abundant in afferent pathways of the spinal cord with higher density in the sacral segments, and may be involved in pain transmission and integration of the sacral autonomic reflex pathways (Zudenigo and Lackovic, 1989; Fahrenkrug, 1993) and intrinsic neurons of the bipolar type in the neocortex (Magistretti, 1990). The nerves containing VIP, which innervate blood vessels, non-vascular smooth muscle, mucosal epithelium and glands, comprise a major and widespread population of the peptide-containing systems (Fahrenkrug, 1993). Nerves immunoreactive to VIP are found in the heart, lung, kidney, spleen, digestive and genitourinary tract, eye, skin, ovaries, lymph nodules, and thyroid gland (Zimmerman et al., 1988; Zudenigo and Lackovic, 1989; Chevendra and Weaver, 1992; Yamamoto et al., 1994) and in the optic, sphenopalatine, submandibular and sublingual ganglia (Gibbins et al., 1984). VIP is found in all areas of the gastrointestinal tract, with very large amounts of VIP in the colon, ileum, and jejunum, and lesser amounts in the antrum, esophagus, fundus, duodenum, and pancreas. Its concentration in the gut is many times that of secretin (Polak et al., 1978; Zdon et al., 1988). VIP concentration in tissue varies with age and physiological conditions. Cha et al. (1995) provided the first morphological evidence for the loss of VIP neurons in the cerebral cortex of the aged rat.

Secretion and release

VIP is released locally from nerve endings by vagal stimulation (Messel et al., 1991) or by depolarizing concentrations of K^+ or Ca^{++} and lipoxygenase metabolites (Magistretti, 1990). In most cases relatively small amounts seem to enter the general circulation. VIP secretion is regulated by neural and humoral factors. Vagal stimulation caused a significant increase in VIP concentration in the jejunum luminal perfusates and portal venous plasma of cat (Blank et al., 1988). Ingestion of a mixed meal and other food components have demonstrable effects on VIP secretion in pig ileum (Harling et al., 1991), rats (Garcia et al., 1990), rabbits (Duggan et al., 1989), and lambs (Reid et al., 1985). These observation were not confirmed by other researchers (Beck et al., 1984; Chijiiwa et al., 1986), however, observation of parallel release of VIP into both the jejunal lumen and portal circulation by identical regulatory mechanisms have been observed and involve a non-muscarinic process which is under cholinoceptive, nicotinic control (Blank et al., 1988). Before feeding, VIP was detected in only 2 of 12 gastric venous plasma samples (5 and 13 pmol/l). During teasing with food there were increments in VIP of 19 ± 4 pmol/l and during feeding of 27 ± 5 pmol/l. VIP concentration in gastric venous plasma rapidly returned to undetectable fasting levels (< 3mol/l) after cessation of sucking (Reid et al., 1985). In contrast, VIP in the intestinal venous plasma did not rise during teasing or upon commencement of sucking but a peak increment of 34 ± 6 pmol/l occurred at 5 min after cessation of feeding in the milk-fed lamb. VIP is released in anticipation of and during sucking from inhibitory neurones involved in relaxation of gastric structures, while intestinal release of VIP is a consequence of entry of digesta into the small intestine (Reid et al., 1985). The small intestinal distension induced the release of VIP on the anal side so that VIP mediates the descending inhibition of the peristaltic reflex in the feline intestine (Sjoqvist and Fahrenkrug, 1987). High extracellular potassium concentrations or carbachol evoked release of endogenous VIP in submucosa segments from distal colon of guinea pig (Reddix et al., 1994) and human colon (Renzi et al., 1991). Duodenal alkalinization by 0.1 M $NaHCO_3$, 0.1 M Na_2CO_3, and 0.025 M NaOH in the rat resulted in maximum increases in VIP of 116, 155 and 147% (Garcia et al., 1990). Release of endogenous VIP from enteric nerves of isolated rat ileum is independent of extracellular calcium (Belai et al., 1987). VIP is released in response to gastric sodium loads

in rabbits on low salt diets and is accompanied by reduced hepatic metabolism of VIP. Gastric sodium loading releases VIP from the intestine and in rabbits on a low sodium diet (Duggan et al., 1989; Hawley et al., 1991), it appears to decrease VIP metabolism by the liver (Hawley et al., 1991). Both low and high gastric distension was accompanied by a sustained increase in VIP secretion in the distal stomach in rats (Schubert and Makhlouf, 1993). Distension (increasing intraluminal pressure by 10 mm Hg) and intraluminal instillation of homologous gallbladder bile, amino acids, 0.1 M HCl, hypertonic NaCl (3,400 mosm/ kg) and hypertonic glucose (1,100 mosm/kg) all increased the release of VIP-like immunoreactivity (VIP-LI) into the venous effluent in parallel in the isolated, vascularly perfused pig ileum. Being the most potent stimulus, bile increased the output of VIP-LI from 3.29 ± 0.19 to 12.68 ± 4.01 pmol/min (Harling et al., 1991). However, intra-ileal administration of a 100-ml test meal, 100 ml of 5% glucose, amino acids, 10% Intralipos, or bile, induced no change in mesenteric VIP (Chijiiwa et al., 1986). Intra-ileal administration of bile or intravenous caerulein produced a significant increase of plasma VIP in the mesenteric vein (Chijiiwa et al., 1986). Significant increases in VIP levels were also found after intraduodenal perfusion with acetic, lactic acids and organic acids in the rat (Sanchez-Vicente et al., 1995). Administration of 5% and 10% ethanol resulted in significant and dose-dependent increases in mesenteric immunoreactive-VIP (Misawa et al., 1990).

Neuropeptide Y (NPY) (1×10^{-7} M) has been shown to inhibit VIP- induced cAMP accumulation in rat pinealocytes (Harada et al., 1992). Perfusion of ileal segments with tetrodotoxin; opioids, Met-enkephalin and dynorphin; and the α_2 -adrenoceptor agonist, BHT920, decreased VIP output into the venous effluent with a concomitant increase in motility. In addition, blockade of nicotinic receptors also reduced VIP output (Fox-Threlkeld et al., 1991a). Pretreatment with atropine completely blocked the bile-stimulated VIP release and significantly inhibited the caerulein-stimulated release of VIP from canine small intestine (Chijiiwa et al., 1986). Fox-Threlkld et al (1991b) suggested that galanin, being colocalized with VIP in some enteric nerves, may inhibit neural releases of VIP and free the muscle from tonic inhibition by VIP.

Receptor and Binding

Two classes of VIP binding sites have been found in bovine thyroid plasma membranes: the first class with high affinity (Kd = 13 nM) and low capacity (6411 sites/pg), and a class with low affinity (Kd = 480 nm) and high capacity (105,300 sites/pg) at 15 °C (Molinero et al., 1985). In bovine, similar to the human (Couvineau et al., 1995), the VIP receptor belongs to a subfamily of G protein-coupled receptors that includes secretin, glucagon and several other receptors (Molinero et al., 1985). The N-terminal extracellular domain of the human VIP receptor contains highly conserved amino acid residues, which are essential for its intrinsic binding activity (Couvineau et al., 1995). Since VIP receptors are coupled to cAMP-generating systems that are amplified by various neurotransmitters such as noradrenaline, histamine and γ-aminobutyric acid (Magistretti, 1990), VIP generally induces a very large increase in the intracellular cAMP level (Molinero et al., 1985) after VIP binding to membrane receptor in bovine thyroid plasma membranes. However, physiological doses of VIP (1 mmol/min) do not stimulate cAMP accumulation in sheep pituitary cells (Sawangjaroen et al., 1996).

With studies of jejuno-ileal epithelial cells from 14-60 day-old rats, the potency and specificity of the VIP receptor-effector system did not vary during development. However, the concentration of VIP receptors and the efficiency of VIP stimulation of cyclic AMP production increased from suckling to adult conditions, and VIP levels in jejuno-ileal tissue followed a parallel course (Fernandez-Moreno et al., 1988). VIP induced a significant and persistent increase in cAMP production, which lasted for 10 min in intestinal epithelial cells isolated during rat development of the rat, from day 17 of gestation to adulthood (Chastre et al., 1987). The intracellular mechanisms that mediate these actions of VIP may involve different transduction cascades triggered by subsets of VIP binding sites that may coexist in the same tissue (Muller et al., 1995). Although VIP exerts many of its effects through stimulation of adenylyl cyclase, there is increasing evidence that other signaling pathways may contribute to its action. Receptor/ Gi3 coupling makes a significant contribution to VIP-mediated signaling in the lung (Diehl et al., 1996). VIP (200 nM) was also found to activate Ca^{++} influx into pituitary cells. The increase in Ca^{++} permeability showed a peak at 5 s and

remained significantly higher than control values for up to 1 min (Pizzi et al., 1990), sustaining VIP-induced prolactin release.

Upon binding to the receptor, VIP is rapidly internalized, probably by a process of receptor-mediated endocytosis. This internalization leads to a decrease in the cell surface receptor number and simultaneously to a homologous desensitization of adenylate cyclase in human lymphocytes B cell (Q'Dorisio et al., 1989). On the other hand, heterologous upregulation of high affinity VIP receptors on mononuclear blood cells takes place during combined strenuous physical exercise, and calorie deficiency (Wiik et al., 1988). With almost continuous physical activity and energy deficiency, the maximum binding capacity for the high affinity VIP receptors on blood mononuclear leucocytes increased from 0.71 fmol/million cells to a maximum of 7.33 fmol/million cells on day 4. Plasma VIP concentration increased from 8.8 pmol/L to a maximum of 23.4 pmol/L on the second day of the course of exercise plus energy restriction (Wiik et al., 1988). These data suggest that there are other factors that may regulate relative affinity of the VIP receptor, various subtypes of VIP receptors in different tissue or cell membranes may have different signalling pathways that result in various responses in different situations.

Degradation

VIP in the circulation is mainly degraded by the liver and lung (Luis et al., 1988; Bernhard et al., 1989). VIP released into the portal circulation by a meal stimulus is rapidly cleared from plasma in liver (Brook et al., 1988). In the lung, the receptors for VIP in the alveolar capillaries are of high affinity and capacity to extract VIP from the circulation. The degradation products are of low molecular weight. The clearance rate was decreased at higher concentrations of VIP (Bernhard et al., 1989). The half time of VIP in plasma is about 1 minute (Domschke et al., 1978; Brook et al. 1988).

In target cells VIP is degraded in the lysosomes, while most of the receptors are recycled back to the cell surface (Luis et al., 1988). Crude membrane fractions prepared from rabbit gastric fundic muscle degraded VIP with an average specific activity of 0.96

nmol/min/mg protein at 37 °C, pH 7.5, and at the initial peptide concentrations = 0.05 mM. The VIP degradation was inhibited by metal chelators EDTA and o-phenanthroline. $CaCl_2$ at 0.3-1.0 mM enhanced VIP degradation up to twofold (Kobayashi et al., 1994).

Biological Function

VIP has been shown to be involved in maturation, growth, and maintenance of neurons (Said and Mutt, 1970; Brenneman et al., 1990; Gozes and Brenneman, 1993). The primary physiological actions may be classified as follows. 1) VIP is involved in the coupling between energy metabolism, blood flow and neuronal activity in bipolar neurones of the cerebral cortex (Fahrenkrug, 1993). VIP provokes the excitation, vasodilatation and together with noradrenaline participates in the regulation of cortical energy metabolism in telencephalic area of central nervous system (McCulloch and Edvinsson, 1980; Zudenigo and Lackovic, 1989). Furthermore, VIP in the brain plays a role in circadian rhythms and melatonin and pituitary hormone secretion (Schomerus et al., 1996). 2) In the peripheral nervous system VIP, as a transmitter of non-cholinergic and non-adrenergic autonomic events, display its biologic effects on the vasodilatation, relaxation of smooth muscle and influences on exocrine gland secretion (Zudenigo and Lackovic, 1989; Fahrenkrug, 1993). 3) in the endocrine system VIP stimulates the secretion of different hormones, such as: prolactin, growth hormone, ACTH, oxytocin, vasopressin, thyroid hormones, insulin and glucagon (Ohneda et al., 1977; Holst et al., 1987; Zudenigo and Lackovic, 1989; Lam, 1991). VIP suppressed food-stimulated release of gastrin and gastrin-stimulated acid secretion from Heidenhain pouch (Villar et al., 1976).

VIP released from intrapancreatic nerve fibers might participate in the parasympathetic control of pancreatic secretion in guinea pig (GP) (Raufman et al., 1986; Zudenigo and Lackovic, 1989). In the ovary VIP seems to play an important role as regulator and/or modulator of folliculogenesis and steroidogenesis. In the male genital tract VIP seems to participate in the control of erection (Ottesen and Fahrenkrug, 1995). VIP appears to be involved in the regulation of a variety of gastrointestinal functions including epithelial ion

transport, gastric secretion, ovine parotid secretion, hemodynamic regulation, immune response, esophageal, gastric and intestinal motility (Zimmerman et al., 1988; Wright et al., 1991). VIP also may regulate the maturation and functions of the intestine and liver during fetal life because specific VIP receptors were expressed before the morphological and functional differentiation of intestinal and liver cells in the rat (Chastre et al., 1987).

VIP of hypothalamic origin acts in an autocrine fashion to stimulate prolactin (PRL) release (Nagy et al., 1988; Reichlin, 1988; Lam, 1991). At doses from 10^{-9} to 10^{-7}M VIP, the amount of GH released was significantly greater than in the controls. Incubation with the media containing 10^{-7}M VIP and combined treatment with VIP plus GHRH increased GH by 40 and 182%, respectively. VIP acts directly on cultured adenohypophysial cells to induce GH release in cattle (Soliman et al., 1995). One potential role of hypothalamic VIP may also involve activation of hypothalamic-releasing factors to regulate ACTH and cortisol levels during or after a meal (Alexander et al., 1995). Additional potential neuroendocrine actions of VIP are in the secretomotor control in the thyroid glands, and in the regulation of somatostatin secretion and synthesis in rat hypothalamic cell culture (De Los Frailes et al., 1991). In contrast, Sawangjaroen and Curlewis (1994) infused VIP into the carotid artery of ewe over a 10 min period, and in no case was an effect on pituitary hormone secretion observed. In dispersed cell cultures (but not in whole hypothalamic slices from adult animals), VIP stimulates somatostatin secretion and independently stimulates the formation of somatostatin mRNA (Reichlin, 1988). The distribution of VIP in both adrenal cortex and medullary tissue suggests an important role in medullary modulation of adrenal cortical function (Holzwarth, 1984). VIP stimulates renin and aldosterone secretion that may modulates sodium excretion by its effects on angiotensin II metabolism (Davis et al., 1992). VIP has been shown to stimulate expression of the NPY gene through activation of cAMP-dependent protein kinase (Colbert et al., 1994).

VIP, as a neurotransmitter, promotes a vasodilation in the cardiovascular system, such as non-cholinergic rectal vasodilatation and coronary vasodilation etc. (Anderson et al., 1983, 1988, 1993; Accili et al., 1995). The locally released VIP might exert a protective effect on the heart because coronary artery occlusion causes a long-term increase in plasma

VIP concentration that decreases after reperfusion when measured in the portal vein and coronary sinus (Gyongyosi et al., 1997). VIP at doses of 2×10^{-12} to 2×10^{-8} mol significantly increased left ventricular pulse, coronary blood flow and myocardial oxygen consumption in dog (Unverferth et al., 1984; Anderson et al., 1988), as well as increased blood flow to the esophagus, pancreas, atria, and ventricles and to the endocardial and epicardial regions of the left ventricular free wall, although blood flow to brain decreased (Unverferth et al., 1984). Suzuki et al. (1995) reported that VIP (0.05, 0.1 & 1.0 nmol) induced significant, time- and concentration-dependent vasodilation increase from baseline values. Arteriolar diameter returned to baseline values within 1-4 min after infusion was stopped. In other research, VIP infusion caused a reduction of splenic perfusion pressure (i.e. vasodilation) and an increase in splenic volume, as well as the vasodilation and relaxation of the capsule (Lundberg et al., 1985). VIP (10^{-14} to 10^{-10} mol/min) caused an atropine resistant vasodilation in submandibular gland. Several hundred fold higher doses of exogenous VIP had to be infused than the amounts of VIP seen in the venous outflow during maximal nerve stimulation at a similar vasodilatory response. Combined infusions of acetylcholine (Ach) and VIP had mostly an additive effect on vasodilation in submandibular gland of the cat (Lundberg et al., 1982). The encapsulation of VIP into liposomes potentiates and prolongs of its vasorelaxant effects in the peripheral microcirculation in vivo (Suzuki et al., 1995). Recent studies showed that VIP microinjection at different locations has different physiological effects. Microinjection of VIP (1-100 pmol) into the dorsal vagal complex in rats (Krowicki et al., 1997) and intracerebroventricular (icv) injection in rats (10 µg)(Shido et al., 1989) increased systemic arterial blood pressure. Mean arterial blood pressure increased in response to VIP injection in the nucleus raphe obscures of the rat at the highest dose of 100 pmol only, but no changes were observed after microinjection of VIP (1-100 pmol) into the region of the nucleus ambiguous (Krowicki et al., 1997).

Chatelain et al. (1983) reported that there are "VIP-preferring" receptors in dog and monkey heart, relative to secretin in cat. Following intravenous administration of VIP (10 µg i.v.) in conscious dog, the heart rate increased by 28.1 %, from 165 ± 8.5 to 209.1 ± 7 beats/min and the mean arterial blood pressure decreased by 37.9%, from 126.6 ± 2.6 to 79.1

± 4.9 mmHg (Roossien et al., 1997). Similar results were reported by Rigel (1988). The increased heart rate resulting from exogenous VIP could be an integrated reflex to lowered blood pressure resulting in increased the heart neural action in association with peripheral vasodilation. Direct chronotropic effects of VIP were also evaluated in five isolated neonatal canine hearts using a modified Langendorff technique. The spontaneous cardiac cycle length decreased from 403 ± 88 to 293 ± 69 ms (-28%) in newborn hearts, after exposure to 0.1-0.5 nmol of VIP (Pickoff et al., 1994). Sinus cycle length and AV nodal conduction time were also shorten by VIP (Pickoff et al., 1994). In vivo, administration of exogenous VIP, 10^{-8} M, increased sinus rate by 20%. This increase in heart rate was reduced significantly to 8% by the VIP antagonist [D-p-Cl-Phe6, Leu17]VIP, 10^{-7} M, which alone had no effect on sinus rate (Shvilkin et al., 1988). In one experiment with conscious sheep, VIP (0.07, 0.2, 0.6, and 1.8 nmol/min) was infused into the carotid artery over a 10 min period. There was a dose-dependent increase in heart rate. At the highest doses tested, pulse pressure and mean arterial pressure tended to increase and decrease, respectively. However, only the decrease in mean arterial pressure following the highest dose of VIP reached significance. At the highest doses tested, heart rate increased nearly threefold during the infusion while mean arterial pressure declined by 18.5% (Sawangjaroen et al., 1992). A similar result was reported in the ewe by Sawangjaroen and Curlewis (1994). In contrast, bolus injections of VIP at dose of 93.7 pmol did not produced significant changes in heart rate, left ventricular pressure, or contractility although VIP caused a dose-dependent decrease in the perfusion pressure of hearts perfused at constant flow, suggesting the presence of one receptor subtype for VIP on rabbit coronary vessels (Accili et al., 1995). Cardiovascular effects of VIP infusion in intact animal are, therefore, complex.

In the lung, there is a high concentration of VIP, which may act as a potent bronchodilator (Bernhard et al., 1989; Nohr et al., 1993; Tang et al., 1995). However, the available data do not indicate the principle role of VIP in respiratory regulation.

Neuropeptides have recently been shown to modulate the immune response. When VIP is released from nerve endings and from immune cells, in vitro, it appears to modulate the mobility and adherence of lymphocytes and macrophages, phagocytic cell functions

(phagocytosis and free radical production), the lymphocyte proliferative response, lymphokine and immunoglobulin production and natural killer cell activity, with opposite effects in vitro on these same immune cell functions (Kimata and Fujimoto, 1994). VIP in a range from 10^{-12} to 10^{-7} M increased significantly the phagocytosis and digestion capacities of rat peritoneal macrophages through PKC activation (De La Fuente et al., 1993). The functional significance of VIP effects on the immune system depends on its ability to help coordinate a complex array of cellular and subcellular events, including events that occur in lymphoid compartments, and in musculature and intramural blood circulation. However, the modulatory role of VIP in immune regulation is not well understood. VIP appears to modulate maturation of specific populations of effector cells, T cell recognition, antibody production, and homing capabilities (Kimata and Fujimoto, 1994). VIP also caused a concentration-dependent relaxation of bradykinin-induced contractions of lymphatic vessels. The threshold and maximum relaxations were achieved with VIP at concentrations less than 6×10^{-9} M and 3×10^{-7} M, respectively (Ohhashi et al. 1983). Downregulation of T lymphocyte proliferation by VIP is possibly through its effect on cytokine expression (Tang et al. 1995). These effects of VIP are tissue-specific and are probably dependent on the resident cell populations within the lymphoid tissue and the surrounding microenvironment. Different microenvironments within the same lymphoid tissue may also influence the modulatory role of VIP. In addition, effects of VIP on immune function may result from indirect effects on secretory cells, endothelial cells, and smooth muscle cells in blood vessels, ducts, and respiratory airways. Presence of VIP has been demonstrated in lymphoid cells, and if released, could exert a paracrine action on central and peripheral lymphoid organs, and might participate in the bidirectional communication between the nervous and the immune systems (Gomariz et al., 1993).

VIP displays its role either independently or co-operatively with other nutritional and neural factors. Even if there is the structural homology to that of the glucagon family, individual characteristics exist in the synergistic effects of VIP on the ACh-mediated secretion of fluid and protein from the rat submandibular gland (Iwabuchi and Masuhara, 1995). However, there is interaction with other agents in secretory glands. An arterial blood

VIP concentration of 1.5 - 2.5 X 10^{-9} mol/L did not evoke fluid secretion in ovine parotid glands. The same blood concentration of VIP potentiated the stimulation of salivary flow rate caused by intraarterial infusion of the cholinergic agent bethanechol (Wright et al. 1991). VIPs from guinea pig (GP), goat, rat and synthetic mammalian VIP are indistinguishable in their inhibition of binding of ^{125}I-labelled synthetic VIP to dispersed acini from GP pancreas, and are also similar in their efficacy and potency in stimulating amylase release from these acini (Raufman et al. 1986). Acetylcholine-mediated (ACh-mediated) secretion of fluid and protein from rat submandibular glands was enhanced by intravenous injection of VIP (Iwabuchi and Masuhara, 1995).

VIP stimulates exocrine pancreatic secretion and conductance directly and indirectly (Blitz et al. 1985; Alonso et al. 1994; Wheeler et al., 1997). After the infusion of VIP a linear dose-response relationship for pancreatic flow rate and bicarbonate output, up to the dose of 4 mg/kg, was observed although VIP did not significantly stimulate pancreatic protein output (Rodriguez-Lopez et al., 1995). Edwards et al. (1997) found that VIP modulated the release of pancreatic polypeptide, but not pancreatic insulin or glucagon in the conscious calf. However, local infusion of an identical dose of VIP into the duodenal arterial circulation significantly increased the volume, protein output and trypsin output of pancreatic juice, but infusion of VIP into the jugular vein for 1 min at dose of 7 pmol /kg/min did not stimulate pancreatic secretion (Kiela et al., 1996). However, VIP at 900 ng/kg of BW did stimulate blood flow on the exocrine pancreas (Jansson, 1994).

VIP is a strong stimulator of lipolysis in adipose tissue from laboratory animals. VIP at a concentration of 10^{-6} mol/l stimulated free fatty acid release. The lipolytic activity of VIP seems to be only of pharmacological interest (Richter et al., 1989) because of high dose required. Therefore, its physiological significance is limited in relation to lipolysis.

VIP is involved in regulation of epithelial secretion and transport as a neurotransmitter within the submucous plexus in the colon of the guinea pig (Reddix et al., 1994), rat (Nassar et al., 1995) and sheep (Hyun et al., 1995). VIP is responsible for at least part of the neurally controlled fluid and bicarbonate secretion from the pig pancreas. Continuous intravenous VIP infusion (11.2 ng/kg/min) throughout an experimental period

of 160 min produced a 60% decrease in alanine absorption and 40% decrease in gastric acid secretion during the second hour of infusion in the rat (Nassar et al., 1995). Hyun et al. (1995) reported that VIP decreased water absorption at 10 and 30 pmol/kg/hr and converted to secretion at over 100 pmol/kg/hr in a dose-dependent manner. Sodium flux changed to secretion only at 1,000 pmol/kg/hr, but chloride flux remained absorptive even at the highest dose of VIP. Bicarbonate secretion was stimulated dose-dependently by VIP. Potassium secretion was also increased at all doses, though this response was not dose-dependent. The net glucose absorption was not altered by VIP at any dose in the jejunal loop of sheep (Hyun et al., 1995). The response to the perfused doses of 0.15 to 2.4 nmol/100g/h of VIP differed qualitatively and quantitatively in the three segments. VIP increased bicarbonate secretion and induced chloride secretion in the duodenum, induced chloride secretion in the jejunum without changing bicarbonate minimal influx, induced bicarbonate secretion and suppressed chloride absorption in the ileum (Chikhlssa et al., 1992).

The prevailing effect of VIP on the gastrointestine is an inhibition of myoelectric activity in monogastric and ruminant animals (Ito et al., 1988; Wechsung and Houvenaghel, 1994). Intravenous infusion of VIP at dose of 150 and 300 pmol/kg/min, both resulted in a decrease in antral electrical activity. The amplitude of the gastric phasic contractions was significantly reduced by a close intraarterial injection of VIP (3 mg/kg). Either intravenous or intra-aortic infusions of VIP inhibited the electrical activity of both the antrum and pylorus for several minutes (Deloof et al., 1988). VIP administration induced relaxations in longitudinal muscle strips of the rat gastric fundus (De Beurme and Lefebvre, 1988). In concious sheep, VIP infused at 1 nmol/min prolonged the quiescent time and decreased spike amplitude of reticulum, omasum and reticulo-omasal orifice (ROO) after 3 to 4 min of infusion and a complete cessation of ROO spiking activity 8 min after commencement of VIP infusion (Okine et al., 1996), similar to results reported by Reid and Titchen (1988). However, microinjection of VIP into the dorsal vagal complex increased intragastric pressure (1-100 pmol) and pyloric smooth muscle contractile activity (100 pmol) in rats (Krowicki et al., 1997), whereas VIP microinjection into the nucleus raphe obscures decreased intragastric pressure and inhibited pyloric smooth muscle contractile activity. In the small

intestine, only the lower dose caused a shortening of the irregular spiking activity (ISA) phase in the jejunum and ileum. In the jejunum VIP resulted in a reduction of the MMC interval (Wechsung and Houvenaghel, 1994). VIP caused a dose-dependent fall in the internal anal sphincter (IAS) pressures by a direct action at the IAS smooth muscle (Nurko and Rattan, 1988). These data indicated that VIP effects on smooth muscle are dependent on the site of administration (central or prepheral).

VIP has been reported to exert effects on body temperature, brown adipose tissue thermogenesis and thermoregulatory behavior. Shido et al. (1989) reported that the intracerebroventricular (icv) injection of VIP at a dose of 10 μg in rats produced hyperthermia with an increase in the positive difference between the interscapular brown adipose tissue and colonic temperatures at ambient temperatures of 18, 23, and 28 °C, but had little effect on nonevaporative heat loss (Kulkosky et al., 1989). Behavioural effects of VIP were observed in rat and hamster. The intracerebroventricular injection of VIP sex-dependently decreased observed resting behavior during 1 h after injections at 0.1-10.0 μg. Grooming behavior was increased in hamsters, rearing and standing behaviours were increased in rats. Drinking behavior was suppressed in rats by VIP at 10.0 μg. Intraperitoneal (IP) VIP (100.0 μg /kg) increased 5% ethanol intake and decreased eating behavior in fluid-deprived male rats. The increase in ethanol intake produced by IP VIP was prevented by IP CCK octapeptide (4.0 μg /kg) in the rat and hamster (Kulkosky et al., 1989). However, chronic ethanol intake in 6-12 week of rats resulted in a decreased of ^{125}I-VIP binding to rat enterocytes (Jimenez et al., 1992). VIP probably controls thermoregulatory and ingestive behaviors through effects in the central nervous system (Kulkosky et al., 1989). It is not clear whether peripheral administration of VIP is likely to have similar effects.

The ROO of ruminants plays a significant role in the regulation of particulate and fluid digesta flow from the rumen to the lower gastrointestinal tract (McBride et al., 1984; Okine et al., 1989; Croom et al., 1990;). The known neurotransmitters involved with regulation of the ROO are acetylcholine, norepinephrine, epinephrine and the peptidergic neurotransmitter, VIP (Newhook and Titchen 1972; Reid and Titchen 1985). The effects of

parasympathetic agonists or antagonists on reticulo-omsal function are poorly understood. Administration of atropine is known to inhibit vagally mediated closure of the orifice (Newhook and Titchen 1972). The administration of cholinergic agonists is known to increase the flow of digesta from the rumino-reticulum (Croom et al., 1990). This effect is due, in part, to an increase in salivary flow; however, an increase in the opening time of the ROO may also be involved. There is a very high density of VIP receptors on smooth muscle surrounding the ROO. Exogenous VIP infusion via a gastric artery catheter induced a longer resting and low amplitude spike of EMG at the ROO (Okine and Mathison, 1996). It was concluded, therefore, that VIP might have extended the ROO open period resulting in facilitation of the digesta outflow from the ruminoreticulum.

Based on reported data, infusion of exogenous VIP may induce very broad physiological responses in whole body of animals. Specific observation on physiological action of VIP may be difficult because responses to VIP treatment are complex. There are several potential ways in which VIP might influence digestion and feed intake. These biological effects could include the release of prolactin, growth hormone and NPY by VIP leading to change in energy transactions to stimulate the hunger drive. Also, relaxation of the ROO and other luminal sphincters by VIP may alter flow dynamics in the gastrointestinal tract. As a logical extrapolation, VIP might have a potential role in the regulation of feed intake. The increase of heart rate from exogenous VIP infusion or injection implies that VIP might also extend an influence on metabolic rate. The effect of gastrointestinal motility and influences of pancreatic secretion and gut luminal epithelial exchange by VIP also might suggest a role for VIP in the modulation of nutrient digestion. However, there is very little systematic data designed to assess the quantitative potential of VIP on intake, digestion and nutrient supply in ruminants. The present studies were carried out to determine the effects of exogenous VIP on intake, nutrient digestion, metabolic rate, and gastrointestinal motility in sheep.

B. Effect of thermal environments on gastrointestinal motility, nutrition and metabolism

Thermal environments have been shown to influence markedly animal physiology and performance (Kennedy and Milligan, 1978; Cowan et al., 1993; Miaron et al., 1995). In applied animal production systems, there are widespread interactions among thermal environments, diet and nutrient supply and physiological status on digestion and metabolism in animals (Horton and Shantz, 1980; Kennedy et al., 1986). Kennedy et al. (1986) reported that there were interactions between diet, dietary form, and the ratio of nitrogenous to energy-yielding metabolites that determine responses of ruminants to cold exposure. Cold exposure has been shown to reduce retention time of digesta in the forestomach and increase ruminal motility, resulting in a depression in the extent of organic matter digestion in the forestomach (Kennedy and Milligan, 1978; Cowan et al., 1993). However, cold exposure appears to have little or no effect on intestinal rate of passage so that there is no change in apparent efficiency of digestion occurring post-ruminally in wethers exposed to cold temperatures (Christopherson, 1989; Kelly et al., 1989). On the other hand, increasing ambient temperatures significantly reduce feed intake (Dahlanuddin et al., 1996). The magnitude of an animal response to change of thermal environment is dependent on their body condition, plane of nutrition and other factors, such as fleece or coat cover. Shearing increase the sensitivity of lambs to cold temperature stress and lambs on a high plane of nutrition are less sensitive to cold than lambs fed on a maintenance diet (Horton and Shantz, 1980). Reduction in ME intake by heat-exposed ewes helps to attenuate increases in rectal temperature during feeding, which, in turn, decreases the size of the increment in heat production in response to feeding (Achmadi et al., 1994).

Metabolic rate

The metabolic rate is regulated by thermal exposure of the animal. During cold exposures, metabolic rate has been shown to be elevated in sheep (Chappel and Hudson, 1978) and in steers (Delfino and Mathison, 1991; Miaron, et al., 1996) and in cows (McGuire et al., 1989), whereas respiratory frequencies and skin temperatures are decreased (Young, 1975). As temperatures fell from -20 to -30 °C metabolic rate increased from 109 to 152 kcal/kg 0.75 daily for Bighorn ewes and 126 to 173 kcal for Bighorn rams (Chappel and

Hudson, 1978). Rectal temperature was lower in lambs kept at - 12.3 °C (Horton, 1981) and 4 °C (Llamas-Lamas and Combs, 1990), compared to thermoneutral temperature. Nicol and Young (1990) also found an increase of metabolic heat production of steers when they were fed cold or frozen turnip bulbs at below 0 °C environmental temperatures.

The exposure to high environmental temperature, on the other hand, increases respiration rate and rectal temperature in the lamb (Llamas-Lamas and Combs, 1990; Bunting et al., 1992) and in the cow (McGuire et al., 1989), and reduces heat production in sheep (Achmadi et al., 1994).

Feed intake and Water intake

Feed intake is stimulated by the cold exposure of animals (Miaron and Christopherson, 1996). The effect of ambient temperatures (5 vs 18 vs 31 °C) on performance traits of ewe lambs include effects on feed intake (Schanbacher et al., 1982). Intakes of DM and N were higher at 6° than at 25°C (Sun et al., 1994). However, Chappel and Hudson (1978) used the simulated winter weather to investigate bioenergetics of Rocky Mountain Bighorn sheep. Their results showed that voluntary feed intake in mid February decreased to 0.55 of that observed in mid October. The different responses to cold exposures in sheep are related to thermal acclimation, species, body condition and dietary form. Cold exposure increased the rate of eating and increased the voluntary intake of chopped hay diets, compared to other diet forms (Kennedy, 1985). Higher intake in the cold may not mean an increase of metabolizable energy intake in all cases (Von Keyserlingk and Mathison, 1993). There are also considerable variations among studies with respect to effects of temperature on feed intake. In one recent experiment that compared the effect of 20 and 0 °C on intake of alfalfa hay cubes in rams, the cold exposure had no significant effect on feed intake (Sano et al., 1994).

Heat exposure reduces DM and OM intakes (McGuire et al., 1989; Martz et al., 1990; Aldrich et al., 1993; Achmadi et al., 1994). When goats were exposed to progressively increased environmental temperature from 25 to 45 °C in 5°C increments with humidity at 35-45%, increasing ambient temperature significantly reduced intake of both lucerne chaff

and lucerne pellets, and increased water intake (Dahlanuddin et al., 1996). Similarly, the increased environmental temperatures (12, 24 and 30 °C) significantly affected the energy intake in goats and sheep (Sanz et al., 1995). Voluntary intake of wheat straw with or without ammonia treatment was lower in the 36 °C (588 g/d) compared to 24 °C (763 g/d) and 4 °C (764 g/d) (Llamas-Lamas and Combs, 1990). In the range of environmental temperatures between 14.5 and 36.8 °C, DM intake declined more rapidly with increasing daily minimum temperature when diets contained lower ADF (Cummins, 1992). In other studies, dry-weight food intake was not related to ambient temperature (Thompson and Thomson, 1977; Guerrini, 1981) although the effect of high ambient temperatures on wet-weight food intake was significant (Guerrini, 1981).

Water intake is affected by the thermal environment. Water consumption decreased with decreasing temperature (Westra and Christopherson, 1976). Thompson and Thomson (1977) further reported that the intake of water was reduced to 55% of the thermoneutral value during mild cold exposure and to 40% of the thermoneutral value during the moderate cold stress. Moreover, water consumption by steers housed at 32 °C was increased by 62% compared with steers housed at 22 °C (Aldrich et al., 1993). The hot-humid exposure decreased water intake by 4%, while the hot-dry exposure increased water intake by 37- 45% (Guerrini, 1981), compared to cool-humid environments.

Effect on Fermentation

Microbial fermentation results in OM digestion in rumen. In animals consuming a fixed intake, any factor that causes a shorter retention time of digesta in rumen reduces the fermentation of organic matter, and as a result, more dietary protein may escape from the rumen to intestinal tract. In addition, the efficiency of microbial growth in the rumen is improved. These are typical response to cold exposure in sheep and cattle. Therefore, the flow and digestion of non-ammonia N (NAN) in the intestine is often increased during cold exposure (Kennedy et al., 1986; Kelly et al., 1989). However, heat stress leads to a reduction in the efficiency of the process of microbial synthesis (Kaiser and Weniger, 1994), a prolonged retention time, and often an increase in ruminal OM digestibility.

Ruminal total volatile fatty acid (VFA) production is reduced in a cold environment in sheep (Kennedy and Milligan, 1978; Kennedy, 1985; Kelly and Christopherson, 1989). The molar proportions of acetic and isovaleric acids were reduced but that of propionic acid was increased in cold exposure (Kennedy, 1985; Kelly and Christopherson, 1989). The shift in the molar proportions of acetate and propionate in rumen in consistent with relative decrease in rumen digestion of cellulose in the cold environment. With increasing temperature, the concentration of short chain fatty acids decreased by 35.5%, the proportion of acetic acid increased by 12.3% (Kaiser and Weniger, 1994), and the mean pH-value of rumen fluid decreased for both roughage and concentrate rations (Weniger and Stein, 1992; Kaiser and Weniger, 1994). In contrast, cold exposure increased the pH of rumen contents (Kennedy, 1985; Kelly and Christopherson, 1989). Rumen ammonia concentration was increased by heat stress in sheep (Weniger and Stein, 1992) and cattle (White et al., 1992), but during cold exposure, mean rumen ammonia concentration was reduced by 20%, while net appearance of NAN across the forestomach increased from 2.7 to 7.3 g/day. This resulted in a 14% increase in flow of NAN from the abomasum (Kelly et al., 1989). Although cold exposure decreased methane production by 20-30% in several studies (Graham et al., 1959; Rogerson, 1960; Blaxter and Wainman, 1961; Kennedy and Milligan, 1978), a contrasting case was reported by Von Keyserlingk and Mathison (1993). The effects of environment on methane production are, therefore, quite variable. Generally, extreme thermal exposure depresses total VFA production but individual short chain fatty acid often respond differentially to thermal stress. Cold stress increases propionate production and heat stress increases acetate production. Moderate heat exposure appears to increase digestibility of crude fiber and NDF in the forestomach.

Gastrointestinal motility

Thermal exposure influences the form and pattern of forestomach contractions (Miaron and Christopherson, 1992; Okine et al., 1995). In steers, frequencies of contraction of the omasum and rumen were both increased to a much greater extent than that of the reticulum during cold exposure (Christopherson, 1989). Kennedy (1985) demonstrated that

the weight of digesta transferred to the duodenum per reticular contraction was substantially reduced in the cold. Cold exposure resulted in greater stimulation of B-sequence compared to A-sequence movements in the rumen (Christopherson, 1989). The duration of the biphasic reticular contraction during resting in steers was significantly reduced at -10 °C (5.4 s) compared with that at 10 °C (5.7 s) and 28 °C (5.7 s) whereas duration and amplitude of biphasic reticular contractions during rumination were elevated at -10 °C (Miaron and Christopherson, 1992).

Digesta flow

There is negative relationship between environmental temperature and digesta flow from the forestomach (Miaron and Christopherson, 1992; Okine et al., 1995). Cold exposure stimulates abomasum emptying. DM, OM and NAN flows to the abomasum were increased during cold exposure (10 °C) (Kelly and Christopherson, 1989). In spite of the changes in forestomach passage rate in ruminants, there was a lack of effect of ambient temperature on intestinal retention time of ^{103}Ru-P (Kennedy et al., 1986) and no depression in intestinal digestibility of diet nutrients (Christopherson, 1989). However, cold exposure decreased the proportion of small particles in duodenal digesta (Chai et al., 1985) and increased the composition and absorption of intestinal amino acids (Kelly et al., 1989). There is evidence for an interaction of diet type and environmental temperature on concentration of different particle size in intestine (Kennedy, 1985). It appears, therefore, that thermal exposure influences motility of the forestomachs to a greater extent than on that of the intestine.

Exposure of closely shorn sheep to cold ambient temperatures usually results in an increase in the rate of contraction of the reticulum and faster passage or digesta into the small intestine (Miaron and Christopherson, 1992). When giving hay ad libitum, cold stress tended to increase fluid flow from rumen (Chai et al., 1985). In cattle, these researchers found that the particulate passage rate constant was higher at -10 °C than at 10 and 28 °C (0.046 vs 0.038 and 0.034/d, respectively) (Miaron and Christopherson, 1992). The fluid passage-rate constant showed a quadratic response to temperature and was inversely related to digestibility. However, some researchers have reported that particle size of rumen digesta and

rumen fluid volume was not significantly affected by cold exposure in animals receiving chopped hay (Chai et al., 1985). Reduced retention time of digesta in the forestomach has been shown in the cold environment by previous investigations. Mean retention times decreased from 38.5 h at 21.2 °C to 32.5 h in the cold (Westra and Christopherson, 1976). Exposure to cold resulted in a decrease in the turnover time of the particulate marker, ^{103}Ru, from 19 h to 10-12 h in the rumen, a decrease in rumen volume and a significant increase in DM and OM which escaped digestion in the stomach (Kennedy and Milligan, 1978). Increase in flow of NAN from the abomasum during 0-2 °C cold exposure was associated with a 63% increase in flow of undegraded feed N and a nonsignificant decrease in microbial NAN. There was a small increase in flow of most amino acids from the abomasum due to cold exposure, with significant effects for lysine, histidine and tyrosine. Disappearances of lysine and tyrosine in the small intestine were increased by the cold environment (Kelly et al., 1989). Therefore, more OM and NAN were apparently digested in the intestines of sheep exposed to cold than in warm at the same feed intake (Kennedy and Milligan, 1978). The efficiency of microbial synthesis in the rumen was increased in cold-exposed sheep, and was related to the amount of N recycled through the rumen NH3 pool from intraruminal sources (Kennedy and Milligan, 1978). In contrast, Kennedy (1985) reported that ambient temperature did not significantly affect retention times of markers of the particulate and liquid phases in rumen digesta; although it caused significant increases in rate of contraction of the reticulum. In a cold environment the shorter retention time of digesta in the stomach compartments may be due, in part, to changes in forestomach motility (Westra and Christopherson. 1976; Kennedy, 1985; Okine and Mathison, 1996), reduced rumen fluid volume (Kennedy and Milligan, 1978; Kennedy et al., 1986; Kelly and Christopherson, 1989) and, in some circumstances, to changes in particle size reduction due to rumination chewing (Gonyon et al., 1979; Welch, 1982).

When environmental temperature was increased from 20 to 30 °C, transit time of cell wall and fibre-rich feeds decreased by 20% (Weniger and Stein, 1992a). Silanikove et al. (1987) observed that the mean retention time of undigested feed in the rumen and in the entire digestive tract was higher in the non-sheltered than in sheltered sheep in the summer.

The mild heat exposure increased the mean retention time (MRT) of ^{51}Cr-EDTA in the reticulo-rumen, and tended to increase the MRT of all three markers in the omasum and abomasum. However, MRT in the whole gastrointestinal tract was unaffected (Faichney and Barry, 1986) because increased MRT in the abomasum and small intestine associated with decreases in MRT in both the rumen and large intestine (Faichney, 1975). Similar results (from 18 to 32 °C) were reported in cattle with ^{144}Ce by Warren et al. (1974). These results indicate that the animal's neuro-endocrinological system may play important role in adjusting to environmental stress by regulating gastrointestinal motility (Faichney and Barry, 1986), The mechanism by which gastrointestinal fluid volume is regulated by temperature exposure is not clear.

Digestion

The extent of the digestive response to thermal exposure is inversely related to the insulation of the animal (Christopherson, 1989; Von Keyserlingk and Mathison, 1993) and the varied digestibility in different thermal environments is associated with different retention times of digesta in the gastrointestinal tract (Warren et al., 1974; Westra and Christopherson, 1976; Kennedy and Milligan, 1978; Christopherson and Kennedy, 1983). The apparent digestibilities of DM, OM and NAN in the rumen were decreased during cold exposure (Kelly and Christopherson, 1989; von Keyserlingk and Mathison, 1993). The amount of NAN digested in the small intestine relative to the amount of organic matter digested in the whole gastrointestinal tract was higher in cold-exposed sheep (Kelly and Christopherson, 1986) due to short retention time in the forestomach. The disappearance of cell-wall constituents (CWC) and ADF in the rumino-reticulum were decreased by 14 to 12%, respectively, during exposure of sheep to a cold environment (Kelly and Christopherson, 1989), but postruminal digestion of CWC and ADF were not affected by cold exposure (Kennedy, 1985; Kelly and Christopherson, 1989). Urea recycling to the rumen on a low-N diet was increased in the cold environment, however, this did not result in an improvement in N retention (Sun and Christopherson, 1988). In addition, some studies found that urinary energy losses increase during cold exposure (Graham et al., 1959; von

Keyserlingk and Mathison, 1993).

Interactions of thermal exposure and feed quality further affect the digestibility of dietary nutrients (Conrad, 1985; von Keyserlingk and Mathison, 1993). Christopherson (1984) reported that cold exposure did not depress the digestibility of red clover and reed canary grass to the same extent as brome grass or alfalfa. The digestibility of all concentrate diets by ruminants is not altered by cold exposure (Kennedy et al., 1982; McBride and Christopherson, 1984). On the other hand, digestibility of diets consisting of mixture of roughage and concentrate (ranging from 50 to 75 % of the diet) is reduced in a cold environment (Christopherson, 1989), and moreover, the digestibility of nitrogen and some macro minerals (P, S, Ca, Mn) may be reduced (Christopherson, 1984). The effect of rising ambient temperatures on apparent digestibility of crude nutrients was strongly influenced by the type of ration. When feeding a straw ration, the apparent digestibility of dry matter increased in direct proportion to the rising ambient temperature, which was mainly due to increased coefficients of digestibility for the fibre fractions (NDF, ADF) of the diet. In contrast, when feeding concentrate-rich rations, the apparent digestibility of all crude nutrients decreased when ambient temperatures rose from 15 to 30 °C and remained unchange when temperatures increased from 30 to 35 °C (Kaiser and Weniger, 1994). DM digestibility values increased proportionally with increasing ambient temperatures in wethers given a straw diet (Kaiser and Weniger, 1994).

Based on nine temperature-humidity combinations, Weniger and Stein (1992a) found significant effects of environmental temperature and relative humidity on digestibility of crude nutrients except for protein. The data did not support a linear effect of any of the climatic factors on digestibility. High values of digestibility were obtained through a combination of 35 °C temperature and 60% relative humidity. A further increase in the environmental temperature tended to reduce the digestibility except for NDF which showed an increase in the digestibility with additional increases in temperature (Weniger and Stein, 1992b). Different response to different ration types indicated that an increasing ambient temperature (30-35 °C) resulted in a higher metabolizability of feed energy in the straw ration (+19%) and consequently in a higher level of metabolizable energy (+13%) whereas

both rations rich in concentrate showed a decrease in energy metabolizability at about 30 °C ambient temperature (Souto et al., 1990; Kaiser and Weniger, 1993).

DM and OM

Nutrient digestibility is affected by long exposure to cold environmental temperature. Kennedy and Milligan (1978) reported that cold exposure at the lower feed intake reduced the apparent digestibility of DM and organic matter (OM) about 0.055 digestibility units. Apparent digestibility of DM and OM was further decreased about 0.03 units with the higher feed intake in the cold. Westra and Christopherson (1976) reported that the digestibilities of DM and ADF were lower when the sheep were at 0.8 °C than at 17.7 °C. DM digestibility was reduced in the cold by 0.18% per °C drop in temperature. However, exposure of sheep receiving pelleted hay or a hay-grain mixture to cold (-6.5 °C to -10 °C) as compared to warm (18 °C) temperatures resulted in reductions in the apparent digestibilities of DM and N which could not be explained by changes in feed intake. In crossbred wethers, digestibility of DM fell by 0.14% for each degree fall in temperature (from 10 °C to 20 °C), and N retention was reduced (Ames and Brink, 1977). Moreover, apparent digestibilities of DM, OM, and fiber were decreased in the cold (4.7 ± 1.7 °C), compared to a warmer temperature (21 ± 1.8 °C) (von Keyserlingk and Mathison, 1993).

Increasing ambient temperature produced variable results. Guerrini (1981) found that feed digestibility increased between 6% and 14% during hot-dry environmental exposure in sheep. In contrast, in another study exposure to high temperatures significantly decreased digestibility of DM in sheep (Bhattacharya and Hussain, 1974). Digestibility of DM at ad libitum intake was lower at 36 °C (54.6%) than at 24 °C (57.5%) or 4 °C (58.6%), respective results at restricted intake were 58.5, 61.0 and 60.7% for sheep (Llamas-Lamas and Combs, 1990). However, Dahlanuddin et al. (1996) did not find a change in the retention time and feed digestibility in sheep under a high temperature environment. The differences among the reported data may be due to breed and age of animals, feed types and environmental factors other than environmental temperatures.

Apparent digestibility of DM was decreased in response to the cold (von Keyserlingk

and Mathison, 1993). Digestibility decreased at rate of 0.2, 0.2 and 0.1 units per degree for shorn sheep, calves and mature cattle respectively (Christopherson, 1989). However, Sun et al., (1994) reported that no significant effect of ambient temperature on digestibility of DM was observed in sheep fed *ad libitum*. In addition, no interaction was observed between types of straw and environmental temperature for DM digestibility when sheep were given diets ad libitum (Liamas-Lamas and Combs, 1990).

As the environmental temperature decreased the apparent digestibility of OM tended to decrease for a wide range of forage-based diets. Apparent digestibility of OM was reduced in the cold by 0.22 digestibility unit per °C in sheep fed barley straw ration (Von Keyserlingk and Mathison, 1993). This is consistent with the result from sheep fed a basal diet of brome grass hay (Kennedy et al., 1982; Kennedy, 1985; Kelly and Christopherson, 1989). Cold exposure did not affect OM digestibility for barley-canola-seed meal diet (Christopherson, 1989), alfalfa or concentrate diet (Kennedy et al., 1982). Collectively, the results reviewed above implicate a diet x temperature interaction. In higher temperatures apparent digestibilities of organic matter, such as, neutral detergent fibre and crude protein on average were significantly increased (Silanikove et al., 1987; Miaron and Christopherson, 1992).

Protein and amino acids

The digestion of protein is depressed less than that of dietary energy by cold exposure. Apparent N digestibility was significantly depressed from 0.62 to 0.59-0.60 for sheep exposed to cold at both high and low intake (Westra and Christopherson,1976; Ames and Brink, 1977; Horton, 1981; Kennedy, 1985). For some diets (brome grass), cold exposure resulted in increased urea nitrogen recycling to the rumen, increased efficiency of rumen microbial protein synthesis and reduced rumen degradation of dietary protein (Christopherson, 1984). Kelly and Christopherson (1986) reported that cold exposure tended to increase amino acid composition of duodenal NAN by 8 to 17% for the chopped diets. When expressed per 100 g of digestible OM, there was an increase in net absorption of lysine, histidine, alanine and tyrosine across the small intestine in the cold (Christopherson, 1989). In contrast, heat exposure increased the digestibility of crude protein in the rumen

(Silanikove et al., 1987; Kelly et at., 1989; Miaron and Christopherson, 1992). When compared with controls, lambs fed high escape CP retained more N when exposed to high temperatures (2.8 vs 3.6 g of N/d) and less N at neutral temperatures (3.3 vs 3.1 g of N/d) (Bunting et al., 1992). However, some studies did not find the effect of ambient temperature (6-25 °C) on digestibility of nitrogen (Weniger and Stein, 1992a; Sun et al., 1994). Net flux of alpha-amino N was reduced 35% by thermal stress treatments compared with the thermal comfort ad libitum intake treatment. Net fluxes of urea N and ammonia N were not affected by thermal exposure in cows (McGuire et al., 1989). In addition, exposure to high temperature (30 °C) significantly decreased digestibility of protein in Awassi wethers given mixed diet of barley hay and barley grain although the retention of N was not changed (Bhattacharya and Hussain, 1974). The difference among the data reported for various studies may be explained by differences in breed and age of animals, feed types and other interacting environmental factors, such as relative humidity.

There appears to be substantial variations among different studies which describe data on the interaction of environmental temperatures and diet on feed digestion. There have not been very detailed studies on the effects of cold exposure on digestion in animals under *ad libitum* feeding condition. Our experiment, therefore, was designed to gain further insight into the digestive and feed intake responses of sheep receiving diets *ad libitum* in thermoneutral and cold environments.

C. Interaction of Water intake and environmental temperatures on metabolism, physiology, and animal performance

Water, making up more than 50% of the animal body weight, plays a very important role in transportation of nutrients and metabolites within the body, and contributes to loss of heat by evaporation and homeostasis of the internal environment. Disturbances of water balance affect various physiological systems and ultimately animal performance. Maximum water intakes on hot days (35°C) have been shown to be twice the average recorded on cooler days (13-25 °C) in sheep (Hadjipanayiotou, 1995). Daily water consumption was greater in summer (3.4 and 2.2 litres for sheep and goats, respectively) than in winter and spring (1.6

and 1.3 litres, respectively) (Hadjipanayiotou, 1995). During a 50 d study to compare different watering intervals, there were differences in daily water intakes for non-pregnant and dry, pregnant, and lactating ewes: 113, 118 and 188 ml/kg$^{0.73}$ for 24 h intervals; 91, 96 and 152 ml/kg$^{0.73}$ for 48 h intervals; and 77, 89 and 112 ml/kg$^{0.73}$ for 72 h watering intervals, respectively (Aganga et al., 1990).

Dehydration of animals often occurs because of exposure to extreme thermal environments, transportation, water-shortages and disorder such as diarrhoea (Purohit, 1979; Robertshaw and Dmi'el, 1983; Dmi'el, 1986). Reductions in the degree of hydration and (or) disturbances of water balance in the microenvironment create stress situations for the animal. These may induce generation of stress metabolites and result in a diffusion barriers associated with hydrophobic materials in membranes and, possibly, reduce enzymic activities in tissue (Beg, 1990). Moderate dehydration in ruminants has been shown to result from deprivation of water for three days under conditions of heat exposure (Macfarlane et al., 1961; Taneja, 1965; Purohit, 1979). The rate of dehydration (meaning loss of body water) in sheep was 87 ml/kg/24 h (Khan and Ghost, 1985). In normal grazing management without drinking water, Marwari sheep almost reached the limit of physiological tolerance by the end of a period of 13 days (Khan and Ghost, 1972). Disturbance of water balance in sheep has been shown to influence the body heat balance (Taneja, 1965; Macfarlane, 1972). During water deprivation in warm and hot environments, the rectal temperature showed variable changes (Purohit, 1979; El-Nouty and Hassan, 1983; Olsson and Dahlborn, 1989; Abdelatif and Ahmed, 1994). The respiratory rates were decreased significantly (about half of initial value), when animals were completely deprived of water, in the case of sheep (Macfarlane et al., 1961; Schmidt-Nielsen et al., 1967; Purohit, 1979; Aganga et al., 1990; Abdelatif and Ahmed, 1994; Ismail et al., 1996) and goats (Olsson and Dahlborn, 1989). Metabolic rate was also found to be decreased by more than 20 % in dehydrated goats (Dmi'el, 1986) and camels (Schmidt-Nielsen et al., 1967). These metabolic responses could have survival value. A lowered metabolic rate prolongs the period of time that an animal can survive without eating under dehydration. Furthermore, water restriction may induce the endocrine responses which influence metabolic activity depending on the thermal environment (Johnson, 1976).

As mentioned, these results suggest that dehydrated sheep, in addition to other species, may also decrease metabolic rate because the low oxygen consumption in a dehydrated animal is an adaptive mechanism to reduce water needs for thermoregulation. However, More (1984) found that watering only every 72 h over a long period (2 years) did not substantially affect metabolism in the adult Chola ewe, as judged by serum Na^+, K^+, Mn^+, P^-, Cl^- and HCO_3^-. In other studies, longer watering intervals increased rectal temperature (Aganga et al., 1990; Ismail et al., 1996) and decreased respiration and pulse rate in sheep (Ismail et al., 1996).

Ismail et al. (1996) reported that water deprivation reduced body weight, and total water and feed intake. The reduction in body weight as a result of dehydration was highest in crossbred (11.2%) followed by Barki (9.5%) and Suffolk (9.1%) sheep and was higher in summer (22.2-30.8 °C)(12.6%) than in winter (8.2-18.7 °C) (7.3%). Meissner and Belonje (1972) reported that during water deprivation for 7 days at a temperature of 44.4 °C the body mass of two breeds (Damara and Mutton Merino wether sheep) fell by about 29% and the daily feed intake and total water excreted in the faeces fell between 97 and 99%, comparing to control. Wilson and Dudzinski (1973) reported that the amount of food eaten for a given volume of water was decreased at higher temperatures. The decrease in total DM intake was 55.0, 47.9 and 41.5% in Barki, crossbred and Suffolk, respectively, and was more pronounced in winter (47.3 $g/kg^{0.82}$ daily) than in summer (38.6 $g/kg^{0.82}$ daily) (Ismail et al., 1996). These short term weight changes were associated with changes in water intake and apparent shifts in body fluid content (McGuire et al., 1989).

Dehydration under hot conditions induced reductions in plasma volume as water is taken up by the tissues in mammals (Schaefer et al. 1990; Ismail et al., 1996) and also increased the secretion of aldosterone and cortisol, presumably in an attempt to maintain biofluid balance (El-Nouty et al., 1988; Schneider, 1990). Progressive increases of packed blood cell volume (PCV) and haemoglobin (Hb) concentration were evident with increased time off feed and water (Ismail et al., 1996) in sheep. Plasma osmolality increased in both sheep and lactating goats (Laden et al., 1987; Dahlborn et al., 1988; Olsson and Dahlborn, 1989; Abdelatif and Ahmed, 1994) in dehydration. Under deprivation of feed for 48 h with water freely available, plasma creatinine was not changed in rams (Naqvi and Rai, 1988).

There is a close relationship between feed intake and water intake. Water intake was significantly increased from 119.7 to 178.7 g/kg$^{0.75}$ as grass hay allowance increased from 65 to 100% of *ad libitum* although water intake per kg DM intake was not affected by dietary treatment in sheep (Lee et al 1995). Water shortage affects feed intake. Bartholomew et al. (1996) found that when drinking water was allowed at 100, 80 or 60% of ad libitum intake for the first 4 d during the dry season in the semi-arid zone of central Mali, the 60% level of water intake reduced hay intake by 23%. Obviously, the results from water restriction in many previous studies have been confounded with reduced feed intake. It is, therefore, not clear whether water restriction by itself influences metabolic rate and other physiological responses. There has also been limited research that focused on the effect of water restriction on physiological parameters during cold temperature exposure.

The objectives of present study were to determine whether water restriction would induce any reaction in addition to that caused by feed restriction on metabolic rate and blood parameters, and to assess possible interactions between water restriction and environmental temperature.

D. General Hypothesis

1. VIP administration will relax the ROO and may enhance digesta passage and voluntary intake in sheep.
2. Cold temperature exposure will enhance digesta passage, voluntary food intake and intestinal supply of protein and amino acids in sheep.
3. VIP and cold temperature may interact to facilitate level of intake and digesta passage in sheep.
4. VIP and cold temperature will increase heat production and energy requirements in sheep.
5. Water restriction will suppress metabolic rate of sheep, contributing to energy conservation. This response will be modified by environmental temperature.
6. Suppression of metabolic rate during fasting will be greater during water restriction than that when water is available.

E. Objective of Thesis

Studies were carried out to determine:

1. The effect of VIP and environmental temperatures on the gastrointestinal motility and metabolic rate in sheep fed alfalfa pellets.
2. The modulation of VIP and environmental temperatures on intake, digesta flow, OM and amino acid digestion in sheep under two types of feeding availability ad libitum.
3. The potential effect of water restriction on metabolic rate and blood parameters under different environmental temperatures.

Table 1-1. The amino acid sequences of vasoactive intestinal peptide (VIP), peptide histidine isoleucine (PHI), secretin in porcine.

AA sequence	1	2	3	4	5	6	7	8	9	10	11	12	13	14
VIP	His	Ser	Asp	Ala	Val	Phe	Thr	Asp	Asn	Tyr	Thr	Arg	Leu	Arg
PHI	His	Ala	Asp	Gly	Val	Phe	Thr	Ser	Asp	Phe	Ser	Arg	Leu	Leu
Secretin	His	Ser	Asp	Gly	Thr	Phe	Thr	ser	Glu	Leu	Ser	Arg	Leu	Arg
(Continued)														
AA sequence	15	16	17	18	19	20	21	22	23	24	25	26	27	28
VIP	Lys	Gln	Met	Ala	Val	Lys	Lys	Tyr	Leu	Asn	Ser	Ile	Leu	Asn
PHI	Gly	Gln	Leu	Ser	Ala	Lys	Lys	Tyr	Leu	Glu	Ser	Leu	Ile	
Secretin	Asp	Ser	Ala	Arg	Leu	Gln	Arg	Leu	Leu	Gln	Gly	Leu	Val	

F. Literature Cited

Abdelatif, A.M., and M. M. M. Ahmed. 1994. Water restriction, thermoregulation, blood constituents and endocrine responses in Sudanese desert sheep. J. Arid Environ. 26: 171-180.

Accili, E. A., A. M. Buchan, Y. N. Kwok, J. R. Ledsome, and J. C. Brown. 1995. Presence and actions of vasoactive intestinal peptide in the isolated rabbit heart. Canadian Journal of Physiology & Pharmacology. 73(1): 134-9.

Achmadi, J., H. Sano, and Y. Terashima. 1994. Metabolic heat production response to feeding and feed intake in heat-exposed sheep given a concentrate or roughage diet. Animal Science and Technology. 65(3): 251-257.

Aganga, A.A., N. N. Umunna, E. O. Oyedipe, and P. N. Okoh. 1989. Influence of water restriction on some serum constituents in Yankasa ewes. Small Ruminant Research. 2: 19-26.

Aganga, A. A., N. N. Umunna, E. O. Dyedipe, P. N. Okoh, and A. O. Aduku. 1990. Response to water deprivation by Yankasa ewes under different physiological states. Small Ruminant Res. 3(2): 109-115.

Aldrich, C. G., J. A. Paterson, J. L. Tate, and M. S. Kerley. 1993. The effects of endophyte-infected tall fescue consumption on diet utilization and thermal regulation in cattle. Journal of Animal Science. 71(1): 164-70.

Alexander, L. D., K. Evans, and L. D. Sander. 1995. A possible involvement of VIP in feeding-induced secretion of ACTH and corticosterone in the rat. Physiology & Behavior. 58(2): 409-13.

Alonso, R. M., A. M. Rodriguez, L. J. Garcia, M. A. Lopez, and J. J. Calvo. 1994. Comparison between the effects of VIP and the novel peptide PACAP on the exocrine pancreatic secretion of the rat. Pancreas. 9(1): 123-8.

Ames, D. R., and D. R. Brink. 1977. Effect of temperature on lamb performance and protein efficiency ratio. J. Anim. Sci. 44: 136-140.

Anderson, F. L., A. C. Kralios, B. Reid, and G. R. Hanson. 1993. Release of vasoactive intestinal peptide and neuropeptide Y from canine heart. American Journal of

Physiology. 265(3 Pt 2): H959-65.

Anderson, F. L., A. C. Kralios, R. Hershberger, and M. R. Bristow. 1988. Effect of vasoactive intestinal peptide on myocardial contractility and coronary blood flow in the dog: comparison with isoproterenol and forskolin. Journal of Cardiovascular Pharmacology. 12(3): 365-71.

Andersson, P. O., S. R. Bloom, A. V. Edwards, J. Jarhult, and S. Mellander. 1983. Neural vasodilator control in the rectum of the cat and its possible mediation by vasoactive intestinal polypeptide. Journal of Physiology. 344: 49-67.

Bartholomew, P. W., B. Traore, H. Nantoume, N. G. Kone, and R. Ly. 1996. Effects of supplementation of molassed natural pasture hay with urea, and of water intake restriction on feed intakes by zebu cattle in the semi-arid zone of Mali. Tropical Animal Health and Production. 28(2): 191-192.

Beck, B., C. Villaume, J. A. Chayvialle, P. Gariot, M. Ulmer, A. Desalme, and G. Debry. 1984. Influence of caloric intake on gastric inhibitory polypeptide, VIP and gastrin release in man. Peptides. 5(2): 403-6.

Beg, M. A. A. 1990. Theoretical approach to life processes: Part V. Postulates of the hypothesis on aging. Pakistan Journal OF Scientific and Industrial Research 33(3): 92-101

Belai, A., V. Ralevic, and G. Burnstock. 1987. VIP release from enteric nerves is independent of extracellular calcium. Regulatory Peptides. 19(1-2): 79-89.

Bernhard, W., E. Bothe-Sandtfort, H. Koop, W. Cassel, and P. Von Wichert. 1989. Degradation of vasoactive intestinal peptide by isolated, ventilated, and perfused rat lungs. European Journal of Clinical Investigation. 19(6): 506-13.

Bhattacharya, A. N., and F. Hussain. 1974. Intake and utilization of nutrients in sheep fed different levels of roughage under heat stress. J. Anim. Sci. 38(4): 877-886.

Blank, M. A., K. Kimura, and B. M. Jaffe. 1988. Regulatory mechanisms in the luminal and portal release of vasoactive intestinal polypeptide during vagal nerve stimulation in the cat. Regulatory Peptides. 23(1): 15-26.

Blaxter, K. L., and F. W. Wainman. 1961. Environmental temperature and the energy metabolism and heat emission of steers. J. Agric. Sci. (Comb.) 46: 81-90.

Blitz, W., H. J. Beijer, and G. A. Charbon. 1985. Cholinergic and pancreatic actions of purified porcine and synthetic vasoactive intestinal peptide (VIP). Archives Internationales de Pharmacodynamie et de Therapie. 277(1): 66-76.

Brenneman, D. E., T. Nicol, D. Warren, and L. M. Bowers. 1990. Vasoactive intestinal peptide: a neurotrophic releasing agent and an astroglial mitogen. Journal of Neuroscience Research. 25(3): 386-394.

Brook, C. W., R. B. Sewell, A. Shulkes, and R. A. Smallwood. 1988. Hepatic metabolism of vasoactive intestinal polypeptide (VIP) in the rat. Regulatory Peptides. 20(4): 311-22.

Bunting, L. D. L. S. Sticker, and P. J. Wozniak. 1992. Effect of ruminal escape protein and fat on nitrogen utilization in lambs exposed to elevated ambient temperatures. Journal of Animal Science. 70(5): 1518-25.

Cha, C. I., E. Y. Lee, Y. I. Lee, and S. H. Baik. 1995. Age related change in the vasoactive intestinal polypeptide-immunoreactive neurons in the cerebral cortex of aged rats. Neuroscience Letters. 197(1): 45-8.

Chai, K., P. M. Kennedy, L. P. Milligan, and G. W. Mathison. 1985. Effects of cold exposure and plant species on forage intake, chewing behaviour and digesta particle size in sheep. Can. J. Anim. Sci. 65: 69-76.

Chakder, S., and S. Rattan. 1995. Distribution of VIP binding sites in opossum internal anal sphincter circular smooth muscle. Journal of Pharmacology & Experimental Therapeutics. 272(1): 385-91.

Chappel, R. W., and R. J. Hudson. 1978. Winter bioenergetics of Rocky Mountain bighorn sheep. Canadian Journal of Zoology. 56 (11): 2388-2393.

Chastre, E., S. Emami, G. Rosselin, and C. Gespach. 1987. Ontogenic development of vasoactive intestinal peptide receptors in rat intestinal cells and liver. Endocrinology. 121(6): 2211-21.

Chatelain, P., P. Robberecht, M. Waelbroeck, P. De Neef, J. C. Camus, A. N. Huu, J. Roba,

and J. Christophe. 1983. Topographical distribution of the secretin- and VIP-stimulated adenylate cyclase system in the heart of five animal species. Pflugers Archiv - European Journal of Physiology. 397(2): 100-5.

Chevendra, V., and L. C. Weaver. 1992. Distributions of neuropeptide Y, vasoactive intestinal peptide and somatostatin in populations of postganglionic neurons innervating the rat kidney, spleen and intestine. Neuroscience. 50(3): 727-43.

Chijiiwa, Y., T. Misawa, and H. Ibayashi. 1986. Evidence of local mechanism involvement in vasoactive intestinal polypeptide release from canine small intestine. Gastroenterology. 90(6): 1877-81.

Chikhlssa, A. R., A. Gharzouli. G. Charpin. M. Descroix-Vagne, and D. Pansu. 1992. Comparison of VIP-induced electrolyte secretion at three levels in rat small intestine. Reproduction, Nutrition, Development. 32(1): 37-45.

Christopherson, R. J. 1984. Effects of cold environment on digesta and feed intake of ruminants. Farming for future, project: 81-0022 (internal reference)

Christopherson, R. J. 1989. In Proceedings of the First International Symposium on Agricultural Technique for Cold Regions, Obihiro, pp. 81-89.

Christopherson, R. J., and P. M. Kennedy. 1983. Effect of thermal environment on digestion in ruminants. Can. J. Anim. Sci. 63: 477-496.

Colbert, R. A., D. Balbi, A. Johnson, J. A. Bailey, and J. M. Allen. 1994. Vasoactive intestinal peptide stimulates neuropeptide Y gene expression and causes neurite extension in PC12 cells through independent mechanisms. Journal of Neuroscience. 14(11 Pt 2): 7141-7.

Conrad, J. H. 1985. Feeding of farm animals in hot and cold environments. Stress physiology in livestock. In Yousef, M. K. (Ed) Stress Physiology in Livestock, Vol. II. pg 205-226. CRC Press, Boca ration, Fla.

Couvineau, A., P. Gaudin, J. J. Maoret, C. Rouyer-Fessard, P. Nicole, and M. Laburthe. 1995. Highly conserved aspartate 68, tryptophane 73 and glycine 109 in the N-terminal extracellular domain of the human VIP receptor are essential for its ability to bind VIP. Biochemical & Biophysical Research Communications. 206(1): 246-52.

Cowan, R.T., R. J. Moss, and D. V. Kerr. 1993. Northern dairy feedbase 2001:2. Summer feeding system. Tropical Grasslands. 27(3): 150-161.

Croom, W.J. Jr., M. A. Froeschtel, and W. M. Jr. Hagler. 1990. Cholinergic manipulation of digestive function in ruminants and other domestic livestock: A review. J. Anim. Sci. 68: 3023-3032.

Cummins, K. A. 1992. Effect of dietary acid detergent fiber on responses to high environmental temperature. Journal of Dairy Science. 75(6): 1465-71.

Delfino,J. G. and G. W. Mathison. 1991. Effects of cold environment and intake level on the energetic efficiency of feedlot steers. Journal of Animal Science. 69(11): 4577-87.

Dahlanuddin, C. J. Thwaites, and M. K. Hill. 1996. Effects of increasing ambient temperature on the intake and digestibility of high- and low-quality feedstuffs in goats. Journal of Animal Physiology and Animal Nutrition. 75(4-5): 185-191.

Dahlborn, K., K. Holtenius, and K. Olsson. 1988. Effects of intraruminal loads of saline or water followed by voluntary drinking in the dehydrated lactating goat. Acta Physiological Scandinavia. 132: 67-73

Davis, R. E., V. Z. Ye, G. J. Macdonald, and K. A. Duggan. 1992. Vasoactive intestinal peptide regulates angiotensin II catabolism in the rabbit. Acta Physiologica Scandinavica. 153(3): 255-61.

De Beurme, F. A., and R. A. Lefebvre. 1988. Vasoactive intestinal polypeptide as possible mediator of relaxation in the rat gastric fundus. Journal of Pharmacy & Pharmacology. 40(10): 711-5.

De la Fuente, M., M. Delgado, M. del Rio, C. Martinez, A. Hernanz, and R. P. Gomariz. 1993. Stimulation by vasoactive intestinal peptide (VIP) of phagocytic function in rat macrophages. Protein kinase C involvement. Regulatory Peptides. 48(3): 345-53.

De Los Frailes, M. T., F. Sanchez-Franco, M. Jesus-Lorenzo, R. M. Tolon, J. I. Lara, and L. Cacicedo. 1991. Endogenous vasoactive intestinal peptide (VIP) regulates omatostatin secretion by cultured fetal rat cerebral cortical and hypothalamic cells. Regulatory Peptides 34(3): 261-274.

Delfino, J. G., and G. W. Mathison. 1991. Effects of cold environment and intake level on the energetic efficiency of feedlot steers. J. Anim. Sci. 69: 4577.

Deloof, S., D. Croix, and G. Tramu. 1988. The role of vasoactive intestinal polypeptide in the inhibition of antral and pyloric electrical activity in rabbits. Journal of the Autonomic Nervous System. 22(2): 167-73.

Diehl, N.L., J. C. Kermode, and S. M. Shreeve. 1996. Direct evidence for functional coupling of the vasoactive intestinal peptide receptor to Gi3 in native lung membranes. Molecular Pharmacology. 50(3): 624-30.

Dmi'el, R. 1986. Selective sweet secretion and panting modulation in dehydrated goats. J. Therm. Biol. 11(3): 157-159.

Domschke, S., W. Domschke, S. R. Bloom, P. Mitznegg, S. J. Mitchell, G. Lux, and U. Strunz. 1978. Vasoactive intestinal peptide in man: pharmacokinetics, metabolic and circulatory effects. Gut. 19(11): 1049-53.

Duggan, K. A., C. M. Hawley, G. J. Macdonald, and S. Shelley. 1989. Sodium depletion decreases hepatic metabolism of vasoactive intestinal peptide in the rabbit. Journal of Physiology. 418: 251-9.

Edwards, A. V., S. R. Bloom, and M. A. Ghatei. 1997. Pancreatic endocrine responses to the peptides VIP and PACAP in the conscious calf. Experimental Physiology. 82(4): 717-27.

EI-Nouty, F. D., and G. A. Hassan. 1983. Thyroid hormone status and water metabolism in Hereford cows exposed to high ambient temperature and water deprivation. Indian J. Anim. Sci. 53(8): 807-812.

EI-Nouty, F. D., G. A. Hassan, T. H. Taher, M. A. Samak, Z. Aboelezz, and M. H. Salem. 1988. Water requirements and metabolism in Egyptian Barki and Rahmani sheep and Baladi goats during spring, summer and winter seasons. J. Agric. Sci. Camb. 111: 27-34.

Fahrenkrug, J. 1993. Transmitter role of vasoactive intestinal peptide. Pharmacology & Toxicology. 72: 354-363.

Faichney, G. J. 1975. The use of markers to partition digestion within the gastro-intestinal

tract of ruminants. Pages 277-291 in I. W. McDonald and A. I. C. Warner, eds. Digestion and metabolism in the ruminant. University of New England Publishing Unit, Armidale.

Faichney, G. J., and T. N. Barry. 1986. Effects of mild heat exposure and suppression of prolactin secretion on gastro-intestinal tract function and temperature regulation in sheep. Australian Joournal of Biological Sciences. 39(1): 85-97.

Fernandez-Moreno, M. D., J. L. Diaz-Juarez, M. A. Fernandez-Gonzalez, F. Sanchez-Franco, and J. C. Prieto. 1988. The vasoactive intestinal peptide receptor-effector system is developmentally regulated in rat jejuno-ileal epithelial cells. Biochemistry International. 16(2): 331-7.

Fox-Threlkeld, J. A., T. J. McDonald, S. Cipris, Z. Woskowska, and E. E. Daniel. 1991a. Galanin inhibition of vasoactive intestinal polypeptide release and circular muscle motility in the isolated perfused canine ileum. Gastroenterology. 101(6): 1471-6.

Fox-Threlkeld, J. E., H. Manaka, Y. Manaka, S. Cipris, and E. E. Daniel. 1991b. Stimulation of circular muscle motility of the isolated perfused canine ileum: relationship to VIP output. Peptides. 12(5): 1039-45.

Garcia, L. J., A. Minguela, A. Montero, J. J. Calvo, and M. A. Lopez. 1990. Duodenal alkalinization releases secretin and vasoactive intestinal polypeptide and stimulates exocrine pancreatic secretion in the anesthetized rat. Digestion. 47(4): 215-25.

Gibbins, I. L., J. E. Brayden, and J. A. Bevan. 1984. Perivascular nerves with immunoreactivity to vasoactive intestinal polypeptide in cephalic arteries of the cat: distribution, possible origins and functional implications. Neuroscience. 13(4): 1327-46.

Gomariz, R. P., M. Delgado, J. R. Naranjo, B. Mellstrom, A. Tormo, F. Mata, and J. Leceta. 1993. VIP gene expression in rat thymus and spleen. Brain, Behavior, & Immunity. 7(4):271-8.

Gonyon, H. W., R. J. Christioherson, and B. A. Young. 1979. Effects of cold temperature and winter conditions on some aspects of behaviour of feedlot cattle. Applied Animal Ethology. 5: 113-124.

Goodall, E.D., and R.M.B. Kay. 1965. Digestion and absorption in the large intestine of the sheep. Journal of Physiology, London 176: 12-23.

Gozes, I., and D. E. Brenneman. 1993. Neuropeptides as growth and differentiation factors in general and VIP in particular. [Review]. Journal of Molecular Neuroscience. 4(1): 1-9.

Graham, N. M., F. W. Wainman, K. L. Blaxter, and D. G. Armstrong. 1959. Environmental temperature, energy metabolism and heat regulation in sheep. I. Energy metabolism in closely clipped sheep. J. Agric. Sci. (Camb.) 52:13.

Guerrini, V. H. 1981. Food intake of sheep exposed to hot-humid, hot-dry, and cool-humid environments. American Journal of Veterinary Research. 42(4): 658-61.

Gyongyosi, M., J. Kaszaki, J. Nemeth, A. Wolfard, L. Mojzes, and A. Farkas. 1997. Myocardial and gastrointestinal release of vasoactive intestinal peptide during experimental acute myocardial infarction. Coronary Artery Disease. 8(6): 335-41.

Hadjipanayiotou, M. 1995. Fractional outflow of soybean meal from the rumen, water intake and ruminal fermentation pattern in sheep and goats at different seasons and age groups. Small Ruminant Research. 17(2): 137-143.

Harada, Y., M. Okubo, K. Yaga, T. Kaneko, and K. Kaku. 1992. Neuropeptide Y inhibits beta-adrenergic agonist- and vasoactive intestinal peptide-induced cyclic AMP accumulation in rat pinealocytes through pertussis toxin-sensitive G protein. Journal of Neurochemistry. 59(6): 2178-83.

Harling, H., T. Messell, S. S. Poulsen, T. N. Rasmussen, and J. J. Holst. 1991. Galanin and vasoactive intestinal polypeptide: coexistence and corelease from the vascularly perfused pig ileum during distension and chemical stimulation of the mucosa. Digestion. 50(2): 61-71.

Hawley, C. M., K. A. Duggan. G. J. MacDonald, and S. Shelley. 1991. Acute but not chronic gastric sodium administration regulates vasoactive intestinal peptide metabolism by the liver. Acta Physiologica Scandinavica. 143(3): 295-8.

Hernandez, D. E., C. Jennes, and S. G. Emerick. 1987. Brain vasoactive intestinal peptide: a potent stimulant of gastric acid secretion. Brain Research. 420(1): 129-34.

Holst, J. J., J. Fahrenkrug, S. Knuhtsen, S. L. Jensen, O. V. Nielsen, J. M. Lundberg, and T. Hokfelt. 1987. VIP and PHI in the pig pancreas: coexistence, corelease, and cooperative effects. American Journal of Physiology. 252(2 pt 1): G182-9.

Holzwarth, M. A. 1984. The distribution of vasoactive intestinal peptide in the rat adrenal cortex and medulla. Journal of the Autonomic Nervous System. 11(3): 269-83.

Horton, G. M. 1981. Responses of shorn and full-fleeced lambs given two levels of feed intake and exposed to warm and cold temperatures. American Journal of Veterinary Research. 42(12): 2151-4.

Horton, G. M. J., and N. S. Shantz. 1980. The effects of fleece length and intake level on lambs exposed to cold temperatures. Journal of Animal Science. 51(Suppl. 1): 369.

Hyun, H. S., T. Onaga, H. Mineo, and S. Kato. 1995. Effect of vasoactive intestinal polypeptide (VIP) on the net movement of electrolytes and water and glucose absorption in the jejunal loop of sheep. Journal of Veterinary Medical Science. 57(5): 865-9.

Ismail, E., G. A.Hassan, A. Abo-Elezz, and H. Abd-El-Latif. 1996. Physiological responses of Barki and Suffolk sheep and their crossbreeds to dehydration. Egyptian Journal of Animal Production. 33(2): 89-101.

Ito, S., A. Ohga, and T. Ohta. 1988. Gastric relaxation and vasoactive intestinal peptide output in response to reflex vagal stimulation in the dog. J. Physiol. 404: 683-93.

Itoh, N., K. Obata, N.Yanaihara, and H. Okamoto. 1983. Human preprovasoactive intestinal polypeptide contains a novel PHI-27-like peptide, PHM-27. Nature (London), 304: 547.

Iwabuchi, Y., and T. Masuhara. 1995. Effects of vasoactive intestinal peptide and its homologues on the acetylcholine-mediated secretion of fluid and protein from the rat submandibular gland. General Pharmacology. 26(5): 961-70.

Jansson, L. 1994. Vasoactive intestinal polypeptide increases whole pancreatic blood flow but does not affect islet blood flow in the rat. Acta Diabetologica. 31(2): 103-6.

Jimenez, J., J. R. Calvo, P. Molinero, R. Goberna, and J. M. Guerrero. 1992. Chronic ethanol intake inhibits both the vasoactive intestinal peptide binding and the associated cyclic AMP production in rat enterocytes. General Pharmacology. 23(4): 607-11.

Johnson, H. D. 1976. Effects of heat on endocrine response of domestic and laboratory animals. Vol. 1:pp.27-41. *In* S.M. Tromp, eds. Progress in Biometeorology: Amsterdam, Swets and Zeitlinger.

Kaiser, D., and J. H. Weniger, 1994. In vivo and in vitro investigations for nutrient digestibility and heat production of ruminants under heat stress and different nutritional levels: III. In vivo investigation-nitrogen-balance, energy-balance and physiological parameters of thermoregulation. Archiv fuer Tierzucht 37(3): 327-337.

Kaiser, D., and J. H. Weniger. 1993. In vivo and in vitro investigations for nutrient digestibility and heat production of ruminants under heat stress and different nutritional levels: I. In vivo investigations: Research background, experimental design, feed and energy intakes. Archiv Fuer Tierzucht. 36(6): 607-619.

Kelly, J. M., and R. J. Christopherson. 1986. Apparent digestibilities of dry matter, and nitrogen in the small intestine of wethers exposed to cold environments. Agriculture and Forestry Bulletin. 65-66; 65th Annual Feeders' Day Report.

Kelly, J. M., and R. J. Christopherson. 1989. The apparent digestibilities of dry matter, organic matter and nonammonia nitrogen in the forestomach, small intestine, and large intestine of wethers exposed to a cold environment. Canadian Journal of Animal Science. 69: 911.

Kelly, J. M., R. J. Christopherson, and R. J. Early. 1989. Apparent digestibility of amino acids and other nitrogenous compounds in the small intestine of wethers exposed to a cold environment. Canadian Journal of Animal Science. 69(4): 921-929.

Kennedy, P. M. 1985. Influences of cold exposure on digestion of organic matter, rates of passage of digesta in the gastrointestinal tract, and feeding and rumination behaviour in sheep given four forage diets in the chopped, or ground and pelleted form. British Journal of Nutrition. 53(1): 159-173.

Kennedy, P. M., and L. P. Milligan. 1978. Effects of cold exposure on digestion, microbial

synthesis and nitrogen transformations in sheep. Br. J. Nutr. 39:105--117.

Kennedy, P. M., R. J. Christopherson, and L. P. Milligan. 1982. Effects of cold exposure on feed protein degradation, microbial protein synthesis and transfer of plasma urea to the rumen of sheep. Br. J. Nutr. 47:521.

Kennedy, P. M., R. J. J. Early, R. J. Christopherson, and L. P. Milligan. 1986. Nitrogen transformations and duodenal amino acid content in sheep given four forage diets and exposed to warm and cold ambient temperatures. Canadian Journal of Animal Science. 66 (4): 951-957.

von Keyserlingk, G. E., and G. W. Mathison. 1993. The effect of ruminal escape protein and ambient temperature on the efficiency of utilization of metabolizable energy by lambs. Journal of Animal Science. 71(8): 2206-17.

Khan, M. S., and P. K. Ghost. 1985. Body-water relationships during water deprivation in desert animals. Indian J. Anim. Sci. 55(3): 169-172.

Kiela, P., R. Zabielski, P. Podgurniak, M. Midura, W. Barej, P. Gregory, and S. G. Pierzynowski. 1996. Cholecystokinin-8 and vasoactive intestinal polypeptide stimulate exocrine pancreatic secretion via duodenally mediated mechanisms in the conscious pig. Experimental Physiology. 81(3): 375-84.

Kimata, H., and M. Fujimoto. 1994. Vasoactive intestinal peptide specifically induces human IgA1 and IgA2 production. European Journal of Immunology. 24(9): 2262-5.

Kobayashi, R., Y. Chen, T. D. Lee, M. T. Davis, O. Ito, and J. H. Walsh. 1994. Degradation of vasoactive intestinal polypeptide by rabbit gastric smooth muscle membranes. Peptides. 15(2): 323-32.

Krowicki, Z. K., N. A. Nathan, and P. J. Hornby. 1997. Opposing effects of vasoactive intestinal polypeptide on gastric motor function in the dorsal vagal complex and the nucleus raphe obscurus of the rat. Journal of Pharmacology & Experimental Therapeutics. 282(1): 14-22.

Kulkosky, P. J., J. S. Doyle, V. I. Cook, G. W. Glazner, and M. A. Foderaro.1989. Vasoactive intestinal peptide: behavioral effects in the rat and hamster. Pharmacology, Biochemistry & Behavior. 34(2): 387-93.

Laden, S., L. Nehmadi, and R. Yagil. 1987. Dehydration tolerance in Awassi fat-tailed sheep. Can. J. Zool. 65(2): 363-367.

Lam, K. S. 1991. Vasoactive intestinal peptide in the hypothalamus and pituitary. [Review] Neuroendocrinology. 53 (Suppl 1): 45-51.

Lee, B. S., Y. H. Chung, S. Y. Paek, G. S. Lee, B. S. Lee, Y. H. Chung, S. Y. Paek, and G. S. Lee. 1995. Effect of feeding levels of grass hay on nutrient utilization and ruminal pH in sheep. RDA Journal of Agricultural Science, Livestock. 37(2): 498-503.

Llamas-Lamas, G., and D. K. Combs. 1990. Effects of environmental temperature and ammoniation on utilization of straw by sheep. Journal of Animal Science. 68(6): 1719-25.

Luis, J., J. M. Martin, A. el Battari, J. Marvaldi, and J. Pichon. 1988. The vasoactive intestinal peptide (VIP) receptor: recent data and hypothesis. [Review] Biochimie. 70(10): 1311-22.

Lundberg, J. M., A. Anggard, and J. Fahrenkrug. 1982. Complementary role of vasoactive intestinal polypeptide (VIP) and acetylcholine for cat submandibular gland blood flow and secretion. Acta Physiologica Scandinavica. 114(3): 329-37.

Lundberg, J. M., A. Anggard, J. Pernow, and T. Hokfelt. 1985. Neuropeptide Y-, substance P- and VIP-immunoreactive nerves in cat spleen in relation to autonomic vascular and volume control. Cell & Tissue Research. 239(1): 9-18.

Macfarlane, W. V., and B. Howard. 1972. Comparative water and energy economy of wild and domestic mammals. Symposium of the Zoological Society of London. 31: 261-296.

Macfarlane, W.V., R. J. H. Morris, B. Howard, McDonald, and O. E. Budtsolsen. 1961. Water and electrolyte changes in tropical Merino sheep, exposed to dehydration during summer. Aust. J. Agric. Res. 12: 889.

Magistretti, P. J. 1990. VIP neurons in the cerebral cortex. [Review] Trends in Pharmacological Sciences. 11(6): 250-4.

Martz, F. A., C. P. Payne, A. G. Matches, R. L. Belyea, and W. P. Warren. 1990. Forage intake, ruminal dry matter disappearance, and ruminal blood volatile fatty acids for

steers in 18 and 32 degrees C temperatures. Journal of Dairy Science. 73(5): 1280-7.

McBride, B. W., L. P. Milligan, and B. V. Turner. 1984. Endoscopic observations of digesta transfer from the reticulo-rumen to omasum of cattle. Casn. J. Anim. Sci. 64(Suppl. 1): 84-85.

McCulloch, J., and L. Edvinsson. 1980. Cerebral circulatory and metabolic effects of vasoactive intestinal polypeptide. American Journal of Physiology. 238(4): H449-56.

McGuire, M. A., D. K. Beede, M. A. DeLorenzo. C. J. Wilcox, G. B. Huntington, C. K. Reynolds, and R. J. Collier. 1989. Effects of thermal stress and level of feed intake on portal plasma flow and net fluxes of metabolites in lactating Holstein cows. Journal of Animal Science. 67(4): 1050-60.

Meissner, H. H., And P. C. Belonje. 1972. Preliminary study on water and electrolyte metabolism during thermal and dehydrational stress in two breeds of sheep. South African Journal of Animal Science. 2(2): 97-100.

Messell, T., B. N. Dunning, S. Sheikh, and J. J. Holst. 1991. Neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP), but not galanin, are autonomic cotransmitters in the porcine pancreas. International Journal of Pancreatology. 10(2): 133-41.

Miaron, J. O. O., and R. J. Christopherson. 1996. Effect of prolonged thermal exposure on heat production, reticular motility, rumen-fluid and -particulate passage-rate constants, and apparent digestibility in steers. Can. J. Anim. Sci. 72(4): 809-819.

Miaron, J. O. O., R. J. Chistopherson, R. T. Hardin, R. Mosenthin, and S. Cosgrove. 1995. The effect of α_2-adrenoceptor stimulation with guanfacin on heat production of restricted fed steers kept at -9, 11 or 28 °C and on *ad libitum* fed steers acclimatised to -19 and 22 °C. J. Therm. Biol. 20: 291-298.

Misawa, T., J. Aramaki, Y. Chijiiwa, T. Kabemura, T. Nasu, and H.. Nawata. 1990. Vasoactive intestinal polypeptide response to ethanol in dogs. Tohoku Journal of Experimental Medicine. 161(1): 49-54.

Molinero, P., J. R. Calvo. R. Goberna, and J. M. Guerrero. 1985. The interaction of vasoactive intestinal peptide (VIP) with isolated bovine thyroid plasma membranes. Biochemical & Biophysical Research Communications. 128(3): 1336-41.

More, T. 1984. Effects of long-term intermittent watering on plasma electrolytes in Chokla sheep under semi-arid conditions. Indian J. Anim. Sci. 54(4): 391-395.

Muller, J. M., V. Lelievre, L. Becq-Giraudon, and A. C. Meunier. 1995. VIP as a cell-growth and differentiation neuromodulator role in neurodevelopment. [Review]. Molecular Neurobiology. 10(2-3): 115-34.

Nagy, G., J. J. Mulchahey, and J. D. Neill. 1988. Autocrine control of prolactin secretion by vasoactive intestinal peptide. Endocrinology. 122(1): 364-6.

Naqvi, S. M. K., and A. K. Rai. 1988. Effect of fasting on some biochemical constituents of blood in Avivastra sheep. Indian J. Anim. Sci. 58: 1079-1081.

Nassar, C. F., L. E. Abdallah, K. A. Barada, S. F. Atweh, and N. E. Saade. 1995. Effects of intravenous vasoactive intestinal peptide injection on jejunal alanine absorption and gastric acid secretion in rats. Regulatory Peptides. 55(3): 261-7.

Newhook, J.C., and D. A. Titchen. 1972. Effects of stimulation of efferent fibres of the vagus on the reticulo-omasal of the sheep. J. Physiol. 222: 407-418.

Nicol, A. M., and B. A. Young. 1990. Short-term thermal and metabolic responses of sheep to ruminal cooling: effects of level of cooling and physiological state. Can. J. Anim. Sci. 70(3): 833-843.

Nohr, D. L., E. Eiden, and E.Weihe. 1993. Coexpression of vasoactive intestinal peptide, calcitonin gene-related peptide and substance P immunoreactivity in parasympathetic neurons of the rhesus monkey lung. Neuroscience Letters. 199(1): 25-8.

Nurko, S., and S. Rattan. 1988. Role of vasoactive intestinal polypeptide in the internal anal sphincter relaxation of the opossum. Journal of Clinical Investigation. 81(4): 1146-53.

O'Dorisio, M. S., B. T. Shannon, D. J. Fleshman, and L. B. Campolito. 1989. Identification of high affinity receptors for vasoactive intestinal peptide on human lymphocytes of B cell lineage. Journal of Immunology. 142(10): 3533-6.

Ohneda, A., S. Ishii, K. Horigome, M. Chiba, T. Sakai, Y. Kai, K. Watanabe, and S. Yamagata. 1977. Effect of intrapancreatic administration of vasoactive intestinal polypeptide upon the release of insulin and glucagon in dogs. Hormone and

Metabolic Research. 9(6): 447-452.

Ohhashi, T., J. A. Olschowka, and D. M. 1983. Jacobowitz DM. Vasoactive intestinal peptide inhibitory innervation in bovine mesenteric lymphatics. A histochemical and pharmacological study. Circulation Research. 53(4): 535-8.

Okine, E. K., and G. W. Mathison. 1996. Effects of vasoactive intestinal polypeptide on electromyographic activity of the reticulo-omasal orifice in sheep at two levels of intake. Journal of Animal Science. 74(7): 1681-8.

Okine, E. K., G. W. Mathison, and R. T. Hardin. 1989. Effects of changes in frequency of reticular contractions on fluid and particulate passage rates in cattle. J. Anim. Sci. 67: 3388-3396.

Okine, E. K., J. O. O. Miaron, S. Cosgrove, and R. J. Christopherson. 1995. Vasoactive intestinal polypeptide inhibits electromyographic activity of the reticulo-omasal orifice in meal-fed sheep in warm and cold environment. Can. J. Anim. Sci. 75:333.

Olsson, K., and K. Dahlborn. 1989. Fluid balance during heat stress in lactating goats. Quart. J. Exp. Physiol. 74: 645-659.

Ottesen, B., and J. Fahrenkrug. 1995. Vasoactive intestinal polypeptide and other preprovasoactive intestinal polypeptide-derived peptides in the female and male genital tract: localization, biosynthesis, and functional and clinical significance. [Review]. American Journal of Obstetrics & Gynecology. 172(5): 1615-31.

Pickoff, A. S., S. N. Wang, A. Stolfi, D. Ramage, N. T. Ross-Ascuitto, D. W. Kydon, and R. J. Ascuitto. 1994. Vasoactive intestinal peptide: electrophysiologic activity in the newborn heart. Pediatric Research. 35(2): 244-9.

Pizzi, M., M. Memo, M. Benarese, E. Simonazzi, C. Missale, and P. F. Spano. 1990. A mechanism additional to cyclic AMP accumulation for vasoactive intestinal peptide-induced prolactin release. Neuroendocrinology. 51(4): 481-6.

Polak, J. M., A. M. J. Buchan, W. Czykowskaw, E. Solcia, S. R. Bloom, and A. G. E. Pearse. 1978. Bombesin in the gut. In Gut hormones, Ed. Broom, S. R., pp. 541-543. Edinburgh: Churchill Livingstone.

Prysor-Jones, R. A., and J. S. Jenkins. 1988. Vasoactive intestinal peptide and anterior

pituitary function. [Review] Clinical Endocrinology. 29(6): 677-88.

Purohit, G. F. 1979. Water metabolism in desert sheep breeds some physiological responses of dehydration and rehydration in pure and cross bred desert sheep. Trans. Isdt. & Ucds. 4(2): 84-88.

Raufman, J. P., J. Eng, B. H. Du, E. Straus, and R. S. Yalow. 1986. Comparison of mammalian VIP bioactivities in dispersed acini from guinea pig pancreas. Regulatory Peptides. 14(1): 93-7.

Rayford, P. L., T. A. Miller, and J. C. Thompson. 1976. Secretin, chlecystokinin and newer gastrointestinal hormones (Review). New England Journal of Medicine. 294(20): 1093-1101.

Reddix, R., A. Kuhawara, L. Wallace, and H. J. Cooke.1994. Vasoactive intestinal polypeptide: a transmitter in submucous neurons mediating secretion in guinea pig distal colon. Journal of Pharmacology & Experimental Therapeutics. 269(3): 1124-9.

Reichlin, S. 1988. Neuroendocrine significance of vasoactive intestinal polypeptide. [Review] Annals of the New York Academy of Sciences. 527: 431-49.

Reid, A. M., A. Shulkes, and D. A. Titchen. 1988. Gastric and intestinal release of vasoactive intestinal polypeptide in the milk-fed lamb. Regulatory Peptides. 12(1): 43-50.

Reid, A. M., and D. A. Tichen. 1988. Atropin-resistent secretory responses of the ovine parotid gland to reflex and direct parasympathetic stimulation. Quarterly J. Exp. Physiol. 73: 413-424.

Renzi, D., P. Mantellini, C.A. Maggi, C. Fucini, and C. Surrenti. 1991. Ruthenium red selectively antagonizes capsaicin-induced release of vasoactive intestinal polypeptide (VIP) from the human colon. Neuroscience Letters. 126(2): 134-6.

Richter, W. O., H. Robl, and P. Schwandt.1989. Human glucagon and vasoactive intestinal polypeptide (VIP) stimulate free fatty acid release from human adipose tissue in vitro. Peptides. 10(2): 333-5.

Rigel, D. F. 1988. Effects of neuropeptides on heart rate in dogs: comparison of VIP, PHI, NPY, CGRP, and NT. American Journal of Physiology. 255(2 Pt 2): H311-7.

Robertshaw, D., and R. Dmi'el. 1983. The effect of of dehydration on the control of panting

and sweating in the black Bedouin goat Caprahircus. Physological Zoology. 56(3): 412-418.

Rodriguez-Lopez, A. M., I. De Dios, L. J. Garcia, M.A. Lopez, and J. J. Calvo. 1995. Dose-response effects of VIP on the rabbit exocrine pancreatic secretion. Comparison with PACAP-27 actions. Revista Espanola de Fisiologia. 51(1): 29-36.

Rogerson, A. 1960. The effect of environmental temperature on the energy metabolism of cattle. J. Agric. Sci. (Camb.) 55: 359.

Roossien, A., J. R. Brunsting, A. Nijmeijer, J. Zaagsma, and W. G. Zijlstra. 1997. Effects of vasoactive intestinal polypeptide on heart rate in relation to vagal cardioacceleration in conscious dogs. Cardiovascular Research. 33(2): 392-9.

Said, S. I., and V. Mutt. 1970. Polypeptide with broad biological activity: isolation from small intestine, science, 169, 1217.

Sanchez-Vicente, C., F. Rodriguez-Nodal, A. Minguela, L. J. Garcia, J. I. San Roman, J. J. Calvo, and M. A. Lopez. 1995. Cholinergic pathways are involved in secretin and VIP release and the exocrine pancreatic response after intraduodenally perfused acetic and lactic acids in the rat. Pancreas. 10(1): 93-9.

Sano, H, H. Takaya, N. Hasemi, and Y. Terashima. 1994. Effects of cold exposure and intravenous glucose infusion on feed intake and plasma insulin response to feeding in sheep. Animal Science and Technology. 65(1): 1-8

Sanz,M. R., I. Prieto, F. Gil, and J. Fonolla. 1995. Environmental temperature: composition and conformation of the carcasses of pre-ruminant kid goats and lambs. Options Mediterraneennes. Serie A, Seminaires Mediterraneens. No.27: 191-195.

Sawangjaroen, K., and J. D. Curlewis. 1994. Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) on prolactin, luteinizing hormone and growth hormone secretion in the ewe. Journal of Neuroendocrinology. 6(5): 549-55.

Sawangjaroen, K., C. R. Dallemagne, R. B. Cross, and J. D. Curlewis.1992. Effects of pituitary adenylate cyclase activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) on the cardiovascular system in sheep. Peptides. 13(5): 1029-32.

Sawangjaroen, K., C. Sernia, and J. D. Curlewis. 1996. Effects of pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal polypeptide on cyclic AMP accumulation in sheep pituitary cells in vitro. Journal of Endocrinology. 148(3): 545-52.

Schaefer, A. L., S. D. M. Jones, A. K. W. Tong, P. Lepage, and N. L. Murray. 1990. The effects of withholding feed and water on selective blood metabolites in market weight beef steers. Can. J. Anim. Sci. 70:1155.

Schanbacher, B. D., G. L. Hahn, and J. A. Nienaber. 1982. Effects of contrasting photoperiods and temperatures on performance traits of confinement-reared ewe lambs. Journal of Animal Science. 55(3): 620-6.

Schmidt-Nielsen, K., E. C. Crawford, A. E. Newsome, K. S. Rawson, and H. T. Hammel. 1967. Metabolic rate of camels: Effect of body temperature and dehydration. Am. J. Physiol. 212: 341-346.

Schneider, E. G. 1990. In water deprivation osmolality become an important determination of aldosterone secretions. News. Physiol. Sci. 5: 197-201.

Schomerus, C., E. Maronde, E. Laestke, and H. W. Korf. 1996. Vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) induce phosphorylation of the transcription factor CREB in subpopulations of rat pinealocytes: immunocytochemical and immunochemical evidence. Cell & Tissue Research. 286(3): 305-13.

Schubert, M. L., and G. M. Makhlouf. 1993. Gastrin secretion induced by distention is mediated by gastric cholinergic and vasoactive intestinal peptide neurons in rats. Gastroenterology. 104(3): 834-9.

Shido, O., Y. Yoneda, and T. Nagasaka. 1989. Changes in brown adipose tissue metabolism following intraventricular vasoactive intestinal peptide and other gastrointestinal peptides in rats. Japanese Journal of Physiology. 39(3): 359-69.

Shvilkin, A., P. Jr. Danilo, P. Chevalier, F. Chang, I. S. Cohen, and M. R. Rosen. 1988. Vagal release of vasoactive intestinal peptide can promote vagotonic tachycardia in the isolated innervated rat heart. Cardiovascular Research. 28(12): 1769-73.

Sidawy, A. N., H. Sayadi, J. W. Harmon, B. Termanini, B. Andrews, R. G. Depalma, and L. Y. Korman. 1989. Distribution of vasoactive intestinal polypeptide and its receptors in the arteries of the rabbit. Journal of Surgical Research. 47(2): 105-11.

Silanikove, N., Z. Holzer, D. Cohen, R. Benjamin, M. Gutman, and A. Meltzer. 1987. Interrelationship between metabolism of tritiated water, 22sodium and dry matter intake in beef cows, fed poultry litter and wheat straw in free choice. Comp. Biochem. Physiol. 88A: 113-118.

Sjoqvist, A., and J. Fahrenkrug. 1987. Release of vasoactive intestinal polypeptide anally of a local distension of the feline small intestine. Acta Physiologica Scandinavica. 130(3): 433-8.

Soliman, E. B., T. Hashizume, S. Ohashi, and S. Kanematsu. 1995. The interactive effects of VIP, PHI, GHRH, and SRIF on the release of growth hormone from cultured adenohypophysial cells in cattle. Endocrine Journal. 42(5): 717-22.

Souto, P. R. L., J. C. Milagres, J. F. C. Silva, and J. F. C. Da-Silva. 1990. Pesquisa Agropecuaria Brasileira. 25(9): 1247-1251.

Sun, S. S., and R. J. Christopherson. 1988. Effect of dietary crude protein content on young lambs in warm and cold environments. Agriculture and Forestry Bulletin. 41-43; 67th Annual feeders' Day Report.

Sun, Y. X., J. Lee, P. M. Harris, E. R. Sinclair, I. D. Shelton, H. T. Blair, and S. N. McCutcheon. 1994. Nitrogen and sulfur metabolism and plasma thyroid hormone concentrations in fleeceweight-selected and control Romney sheep at two ambient temperatures. Australian Journal of Agricultural Research. 45(2): 339-354.

Suzuki, H., Y. Noda, S. Paul, X. P. Gao, and I. Rubinstein. 1995. Encapsulation of vasoactive intestinal peptide into liposomes: effects on vasodilation in vivo. Life Sciences. 57(15): 1451-7.

Taneja, G. C. 1965. Effect of varying frequency of watering during summer on the rectal temperature, respiration, body weight and packed cell volume of blood of sheep. Indian J. Exp. Biol. 3:259.

Tang, H., A. Welton, and D. Ganea. 1995. Neuropeptide regulation of cytokine expression:

effects of VIP and Ro 25-1553. Journal of Interferon & Cytokine Research. 15(11): 993-1003.

Thompson, G. E., and E. M. Thomson. 1977. Effect of cold exposure on mammary circulation, oxygen consumption and milk secretion in the goat. J. Physiol. 272: 187-196.

Unverferth, D. V., T. M. O'Dorisio, W. W. Muir, J. White, M. M. Miller, R. L. Hamlin, and R. D. Magorien. 1984. Effect of vasoactive intestinal polypeptide on the canine cardiovascular system. Journal of Laboratory and Clinical Medicine. 106(5): 542-550.

Villar, H. V., H. R. Fender, P. L. Rayford, S. R. Bloom, N. I. Ramus, and J. C. Thompson. 1976. Suppression of gastrin release and gastric secretion by gastric inhibitory polypeptide (GIP) and vasoactive intestinal polypeptide (VIP). Annals of Surgery. 184 (1): 97-102.

Wakade, T. D., M. A. Blank, R. K. Malhotra, R. Pourcho, and A. R. Wakade. 1991. The peptide VIP is a neurotransmitter in rat adrenal medulla: physiological role in controlling catecholamine secretion. Journal of Physiology. 444:349-62.

Warren, W. P., F. A. Martz, K. H. Assay, E. S. Hilolerbrand, C. G. Payne, and J. R. Vogt. 1974. Digestibility and rate of passage by steers fed tall fescue, alfalfa and orchard grass hay in 18 and 32 °C ambient temperature. J. Anim. Sci. 39: 93-96.

Wechsung, E., and A. Houvenaghel. 1994. Effect of vasoactive intestinal polypeptide on porcine gastrointestinal electrical activity. Peptides. 15(8): 1373-6.

Welch, J. G. 1982. Rumination, particle size and passage from the rumen. J. Anim. Sci. 54 (4): 885-894.

Weniger, J. H., and M. Stein. 1992a. Influence of environment temperature and humidity on feed digestion in sheep: 1st Communication: Problem performance of the investigation, digestibility. Zuchtungskunde. 64(2): 148-155.

Weniger, J. H., and M. Stein. 1992b. Influence of environment temperature and humidity on feed digestion in sheep: 2nd Communication: digestibilities, investigations and

digestive physiology. Zuchtungskunde. 64(2): 156-165.

Westra, R., and R. J. Christopherson. 1976. Effects of cold on digestibility, retention time of digesta, reticulum motility and thyroid hormones in sheep. Canadian Journal of Animal Science. 56(4): 699-708.

Wheeler, S., J. E. Eardley, K. F. McNulty, C. P. Sutcliffe, and J. D. Morrison. 1997. An investigation into the relative merits of pituitary adenylate cyclase-activating polypeptide (PACAP-27) and vasoactive intestinal polypeptide as vagal neuro-transmitters in exocrine pancreas of rats. Experimental Physiology. 82(4):729-47.

White, T. W., L. D. Bunting, L. S. Sticker, F. G. Hembry, and A. M. Saxton. 1992. Influence of fish meal and supplemental fat on performance of finishing steers exposed to moderate or high ambient temperature. J. Anim. Sci. 70(11): 3286-3292.

Wiik, P., P. K. Opstad, S. Knardahl, and A. Boyum. 1988. Receptors for vasoactive intestinal peptide (VIP) on human mononuclear leucocytes are upregulated during prolonged strain and energy deficiency. Peptides. 9(1):181-6.

Wilson, A. D., and M. L. Dudzinski. 1973. Influence of the concentration and volume of saline water on the food intake of sheep, and on their excretion of sodium and water in urine and faeces. Australian Journal of Agricultural Research. 24 (2): 245-256.

Wright, R. D., J. R. Blair-West, A. P. Gibson, and A. Shulkes.1991. Evidence against the involvement of vasoactive intestinal peptide in ovine parotid secretion and blood flow. Clinical & Experimental Pharmacology & Physiology. 18(7): 469-74.

Yamamoto, Y., N. Kitamura, J. Yamada, and T. Yamashita. 1994. Immunohistochemical study of the distributions of the peptide- and catecholamine-containing nerves in the omasum of the sheep. Acta Anatomica. 149(2): 104-10.

Young, B. A. 1975. Temperature-induced changes in metabolism and body weight of cattle (Bos taurus). Canadian Journal of Physiology & Pharmacology. 53(5): 947-53.

Zdon, M. J., T. E. Adrian, and I. M. Modlin. 1988. Gastric somatostatin release: evidence for direct mediation by calcitonin gene-related peptide and vasoactive intestinal peptide. Journal of Surgical Research. 44(6): 680-6.

Zimmerman, R. P., T. S. Gates, C. R. Mantyh, S. R. Vigna, C. G. Boehmer, and P. W.

Mantyh. 1988. Vasoactive intestinal peptide (VIP) receptors in the canine gastrointestinal tract. Peptides. 9(6):1241-53.

Zudenigo, D., and Z. Lackovic. 1989. Vasoactive intestinal polypeptide: a potential neurotransmitter. Lijecnicki Vjesnik. 111(9-10): 354-9.

CHAPTER TWO

EFFECT OF VIP AND ENVIRONMENTAL TEMPERATURES ON FORESTOMACH ELECTROMYOGRAPHIC ACTIVITY AND GASTROINTESTINAL CONTRACTIONS IN SHEEP

A. Introduction

Studies to date indicate that cold exposure reduces retention time of digesta in the forestomach and increases ruminal motility, resulting in a depression in the extent of organic matter digestion in the forestomach (Kennedy and Milligan, 1978; Christopherson 1984; Cowan et al., 1993). However, cold exposure has little effect or no effect on intestinal rate of passage so that there is no change in apparent efficiency of digestion occurring post-ruminally (Christopherson, 1989). The mechanism by which thermal stress alters rate of passage and digestive activity remains unclear.

Gastrointestinal hormones, such as vasoactive intestinal polypeptide (VIP), cholecystokinin and secretin, have been demonstrated to act on pancreatic secretion and to regulate gut motility. VIP acts as a neurotransmitter in both the central and peripheral nervous systems (Said 1984; Reid et al., 1988a,b; Luts and Sundler, 1994). Studies have shown that nerve fibres containing VIP are located throughout the muscular tissue of the ruminant forestomach and there is a particularly high density in the region of the reticulo-omasal orifice (ROO) (Wathuta, 1986) and in the inner muscle layer of the omasal wall (Yamamoto et al., 1994).

Vasoactive intestinal polypeptide causes a concentration-dependent reduction of acetylcholine-induced isotonic contraction of muscle strips (Denac et al., 1987). Vasoactive intestinal polypeptide was more effective in the muscle strips from calves than those from adult cattle (Denac et al., 1987) although Toullec et al., (1992) did not observe effects of age, weaning or feeding on plasma VIP. Moreover, the circular muscle strips from the reticular groove were more sensitive to VIP than the longitudinal muscle strips from the reticulum

(Denac et al., 1990). Vasoactive intestinal polypeptide also induced a concentration-related relaxation of muscle strips from the calf ROO. Furthermore, both circular and longitudinal muscle strips from the omasal canal of the calf were relaxed by VIP. The relaxing effect of VIP seems to be mediated by VIP receptors on smooth muscle cells (Denac et al., 1990).

Infusion of VIP produced cessation of electromyographic (EMG) activity of the ROO in lambs (Reid and Titchen, 1984, Reid et al., 1988a,b; Okine et al., 1996). As well, VIP reduced the frequency of reticular EMG of sheep prior to, during and after feeding in a warm, but not in a cold environment. Vasoactive intestinal polypeptide increased the mean duration of the ROO quiescence within each cycle to 13 sec in the cold, compared to 9 sec in the warm (Okine et al., 1995). In fasted and milk-fed lambs, the inhibition of EMG activity of the ROO was associated with each biphasic reticular EMG burst (Reid and Titchen, 1988). However, the inactivity of the ROO (relaxation) was not coincidental with the reticular EMG bursts in meal-fed sheep receiving solid diets (Okine et al., 1996). So far, no data are available to clarify the relation of ROO EMG activity and the diameter change of ROO (ROO opening), although a change of ROO activity may help to regulate the outflow of ruminal digesta. It seems likely, therefore, that the relaxation effect of VIP on gastrointestinal muscle (Haraha et al., 1986), and especially on the ROO, could affect digesta flow. Unfortunately, the reported data do not provide a comprehensive assessment of simultaneous changes in EMG activity and movement and pressure responses to VIP in ruminants. Therefore, further research on the physiological effect of VIP is needed to assess its role in regulating gastrointestinal motility.

The objectives of these studies were to investigate (a) the timing of EMG patterns in different forestomach sites, (b) the effect of exogenous VIP on the EMG activity and motility of forestomachs and contraction of various parts of gastrointestinal tract, and (c) effects of moderate environmental temperature changes on EMG activity and the motility of forestomach regions in sheep.

B. Experimental Procedures

Animal Trial Procedures

In a series of four cross-over design experiments, sheep with 40-60 kg body weight were housed in metabolic crates and fed a diet of alfalfa pellets (estimated ME 9.4 MJ/kg, CP 13.4%) ration *ad libitum*. All sheep were given free access to water and iodized salt. Animals were shorn one week before each treatment period. The surgical and experimental procedures used were approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta and animals were cared for under the guideline's of the Canadian Council of Animal Care (CCAC, 1993).

Experiment 1 (exp.1): Four sheep were housed in a thermoneutral environment (21 ± 2 °C) and fed *ad libitum* for 8 h per day. All sheep were surgically fistulated with ruminal and "T" type duodenal cannulas (Kennedy, 1985), and three pairs (O.D. 0.28 mm) electrodes of fluorocarbon-coated stainless steel wires were implanted into the muscle layers of the reticulum, ROO and omasum on the hepatic aspect of these organs as described by Okine et al. (1995). A modified inductive displacement gauge (movement sensor) (Kingma et al., 1980) was made of two parallel insulated copper wires (O.D. 0.3 mm) which were pull through a silastic tubing (I.D. 0.8mm) to form a loops (O.D.10 mm) (Fig. 2-1) and extending wires were covered by OD 1.8mm silicone tubing and the silicon glue was injected into spare space in the tubing to hold parallel wires tightly and all joints were sealed by silicone glue. The movement sensor was anchored on the outside wall of the ROO at the circular direction of the lumen. At the same time, one sterilised polypropylene catheter (I.D. 0.58 and O.D. 0.96 mm) was inserted about 15 cm proximally into the left gastric artery from an arterial branch cannulated mid-way along the lesser curvature of the abomasum (Okine et al., 1995; Okine and Mathison, 1996) for local infusions of saline and VIP (purity 99%; Sigma Chemical Co Product # V3628, ST. Louis, MO) solution. The extension wires of all electrodes and the movement sensor were exteriorized through the body wall with aid of a trocar and soldered to nine pins of a 12 pin nylon-insulated connector. One wire was left free in the body cavity to serve as a ground electrode. The dead space of the catheters was filled with about 2 ml of a sterile physiological saline solution containing procaine penicillin (400 IU ml^{-1}) and heparin (40 IU ml^{-1}) and capped with sterile injection caps. Catheters were subsequently flushed twice daily with 3 ml of 20 IU ml^{-1} heparinized saline solution. When

not being used for measurements and infusions, the wires and tubes were placed in plastic bags sutured to the fleece on the dorsal aspect of the sheep. Antibiotic therapy after surgery was i.v. injection with long acting Liquamycin (1 ml 20^{-1} kg of BW). The surgery was performed at least 14 d before treatments started.

VIP treatment: During experimental periods, VIP (1 nmol/ml^{-1} at a rate of ml min^{-1}) or saline solution (control, at a rate of ml min^{-1}) was infused through the gastric artery for 10 minutes each hour for 3 h on each of 4 consecutive days starting at 9:00 am, 1-2 min before feeding.

Electromyographic and ROO movement sensor recording: To obtain EMG recordings, three pairs of electrodes and movement sensor output wires from each sheep were connected by a cable to the different inputs of four channels of a MP100 EMG data taker of Biopac Systems (BIOPAC System, Inc. Goleta, CA). Data takers were set to a gain of 1000 and sampling rate at 5 reading per second. The voltage input of the movement sensor of ROO was generated by a Function Generator 26202-00 (Cole-Parmer Instrument Company, Niles, Illinois 60714) at 9 volt and 1 mHz. Downward excursion of the recording from the movement sensor indicated a decrease of ROO diameter (closure) whereas upward excursion indicated an increase of ROO diameter (opening). The active duration of EMG in each cycle was measured from the point of small burst appearance to the time that the signal curve returned to baseline. The quiescent period means that period where none myoelectric signal was detected besides the baseline.

Experiment 2 (exp.2): Four sheep from exp.1 were offered feed *ad libitum* for 24 h per day and were housed in a cold environment (1 ± 1.5 °C). Animal preparation and surgery procedure and cannulation was the same as described for exp.1. EMG activities of reticulum, ROO, and omasum and ROO movement were measured by the same equipment and settings as described for exp.1. During treatment periods, VIP (1 nmol min^{-1}) or saline solution (control) was infused through the gastric artery for 10 minutes each hour for 3 hour on each of 4 consecutive days starting at 9:00 am, 1-2 minutes before feeding.

Experiment 3 (exp.3): A complete two factorial cross-over experimental design used four sheep. Animals were offered alfalfa pellets for 24 h per day and housed in metabolic

crates first in a thermoneutral environment (21 ± 2 °C) and then in a cold environment (1 ± 1.5 °C). The preparation of cannulation and catheterizion procedures of the animals was the same as described for exp.1. EMG activities of reticulum, ROO, and omasum and ROO movement were measured by the same equipment and settings as described for exp.1. During treatment periods, VIP (1 nmol min^{-1}) or saline solution (control) was infused through the gastric artery for 10 minutes each hour for 3 hour on each of 4 consecutive days starting at 9:00 am, 1-2 minutes before feeding.

Experiment 4 (exp.4): Six sheep were fed alfalfa pellets for 8 h per day and housed in a thermoneutral environment (21 ± 2 °C). Animals were fistulated with ruminal, and "T" type duodenal and terminal ileal cannulas, and a gastric-artery catheter as described in exp.1. During measurement three pressure sensors were put into the rumen, duodenum and ileum lumen through the respective cannulae 10 min before the measurements. Each pressure sensors consisted of a small balloon (made of the elastic finger part of a medical glove) on the end of a polyethylene tubing. The water-filled balloon was connected to a pressure transformer (Model 1050BP, UFI, Morro Bay, CA) through the sealed polyethylene line (O.D. 2.5 mm). The water volume in the whole system was adjusted to keep a proper inside pressure to sensitively detect pressure changes in the gastrointestinal lumen. The transformed electrical signal was received by the TSD104-MP100 ECG data taker (Biopac Systems, Inc. Goleta, CA) and recorded by the AcqKnowledge system (Biopac Systems, Inc.). Data takers were set to a gain of 2000 for obtaining an optimal signal. The duration of pressure period was measured from the point that pressure signal starts to rise over the baseline to the point that pressure signal totally returns to the baseline. The maximum or minimum pressure duration was the longest or the shortest pressure duration during each of recording periods for 10 min.

In experimental periods, VIP (1.5 nmol min^{-1}) (Purity 97.7%, Peptide Institute, Univ. of Alberta, Edmonton, AB, Canada) or saline (control) was infused at 10 min each 30 min for 3 hours on each of 7 consecutive days starting at 2 min before feeding.

Statistical Analysis

Electromyographic and pressure data were expressed by terms of frequency (spike bursts per min), active duration (burst duration per cycle) and quiescence (time of no activity per cycle). The mean value for each parameter averaged over 10 min periods of infusion treatments in each experiment designed in cross-over model was analyzed by the GLM procedure of SAS (1996). The SAS model for exp.1 and exp.2 included infusion treatment, animals, and their interactions. Data from ROO movement sensor was only analyzed by the paired T-test procedure of SAS because we only obtained data from two animals. The SAS model for exp.3 included infusion treatment, environmental temperatures, animals, and their interactions. Pressure data averaged over 10 min periods of VIP or saline infusion from cross-over design of exp.4 were statistically analysed by paired-T procedure of SAS (1996) to exclude animal effect.

C. Results

EMG Activity Patterns

Recording's prior to feeding show the basic timing of activity in the reticulum, omasum and ROO (Fig. 2-2). The general circadian pattern of EMG activity in the reticulum, ROO and omasum is such that these events occur almost at the same rhythm in each of the three sites, but with some differences in duration. ROO EMG had the longest active duration, omasal EMG active duration was intermediate and the reticular EMG had the shortest active duration in each cycle. Reticular EMG activity showed a specific biphasic character (Fig. 2-2). EMG activities of all three sites of recording were low and had a longer active duration and quiescence before feeding than that after feeding (Table 2-1). As the regular feeding time approached, EMG activity rapidly increased to the levels observed during and after feeding.

Contraction of the reticulum was usually biphasic. The first phase was longer in duration than the second phase and the first phase generally lower in amplitude than the second phase. Within 3 h after feeding the EMG records indicated that there were numerous triphasic contractions and occasionally quartophasic contractions that were usually associated with rumination and eructation. During eating monophasic reticular EMG patterns were also often seen. The percentages of mono-, bi-, tri- or quarto-phasic contractions were 10.0, 69.8,

16.7 and 3.3%, respectively, during the 3 h period after feeding. Monophasic EMG activity had a mean duration of 1.2 ± 0.25 s. The duration of the first phase of biphasic reticular EMG activities was 1.51 ± 0.31 s and of the second phase 0.38 ± 0.06 s. Average interval time between the two bursts of each reticular biphasic activity was 1.4 ± 0.26 s (0.85-3.65 s). The duration of tri-phasic EMG activities were 1.34 ± 0.14 s, 0.40 ± 0.09 s and 0.36 ± 0.08 s for the first, the second and the third peaks, respectively. The duration of quarto-phasic EMG acitivities were 1.23 ± 0.07 s, 0.41 ± 0.11 s, 0.34 ± 0.08 s and 0.25 ± 0.07 s for the first, the second, the third and the fourth peaks, respectively. In our observation the duration of the first part of the biphasic EMG activity gradually increased with time postprandially.

EMG activity of the ROO showed a continuous activity with a very short quiescence (Fig. 2-2). Most of ROO EMG activity had a burst immediately after the first phase spike of reticular EMG activity, but ROO EMG become relatively quiet before the second phase spike of reticular EMG activity. Data from the ROO movement sensor showed that regular closure of the ROO coincided with the first peak of reticular biphasic activity and opening followed after the second peak of reticular biphasic activity. At this time the omasum is in relative relaxation or low EMG activity. Irregular closure and opening of the ROO may also happen 0-2 times between reticular cycle contractions (Fig. 2-3). Our data showed that the ROO is lightly relaxed most times during the quiescence of the reticulum with one or more occurrences of wider opening. However, diameter change of the ROO was not closely related to EMG activity of the ROO because no closure signal coincided with the whole active duration of the ROO EMG.

EMG activity bursts of the omasum preceded those of the reticulum and typically omasal EMG activity ended 0.1-2.6 s before the start of the second biphasic reticular contraction, and then was followed by a long quiescent time (Fig. 2-2). The active duration of omasal EMG activity varied in an irregular fashion, occasionally remaining active over periods of four reticular contractions in spite of an arrest of the second phasic contraction of the reticulum with lower active amplitude.

Effect of VIP

In almost all cases for both saline and VIP infusion, commencement of the infusion initially stimulated an increase of myoelectric spike activity for up to 1 min. In all periods of VIP infusion a transient inhibitory effect was seen 4 min after the start of VIP infusion, and some of these inhibitions lasted to 1.5 min after stopping VIP infusion (Fig. 2-4). When values from the duration of 3-8 min periods of infusions were averaged, VIP significantly ($P < 0.05$) inhibited the EMG activities of the reticulum, ROO and omasum (Fig. 2-5).

In exp.1, VIP infusion did not ($P > 0.05$) affect EMG activities of the reticulum and ROO regarding burst frequency, active duration and quiescence when values were averaged over the 10 min infusion periods (Table 2-2), compared to saline infusion. However, VIP significantly ($P < 0.05$) increased quiescence of the omasum although there were no effects on burst frequency and active duration of the omasum, compared to that of saline.

Two of four ROO movement sensors functioned normally in this experiment. During VIP infusion, the duration (5.74 ± 1.47 s) of opening of the ROO after reticular contraction was significantly ($P < 0.05$) increased, comparing to its duration (5.13 ± 1.65 s) during saline infusion. ROO closure duration (1.46 ± 0.21 s) coinciding with the first phasic contraction of reticular biphasic contraction did not change in response to VIP infusion.

In exp.2, VIP significantly decreased ($P < 0.05$) the frequency of EMG activities and increased ($P < 0.05$) quiescence of EMG activity on the reticulum, but there was no effect on active duration of reticular EMG activity (Table 2-3). VIP did not significantly influence EMG activities of the ROO on all parameters even though there was big numerical difference between VIP and saline infusion. There were significant ($P < 0.05$) effects of VIP on active duration and quiescence of omasal EMG activities. VIP decreased active duration by 77.5% and increased quiescence by 171.1% on omasal EMG activity, comparing to that of saline treatment. However, VIP did not ($P < 0.05$) significantly decrease burst frequency of omasal EMG, compared to that of saline.

Unfortunately, no consistent data was obtained from ROO movement sensor in exp.2 because the sensor may have detached from its original position and one of them was broken wires when animal had exercise at the floor pen during interval of treatment periods.

In exp.3, VIP infusion did not inhibit EMG activity on the reticulum, ROO and

omasum although number values, comparing to that of saline infusion, showed that VIP had some inhibitory tendency (Table 2-4). Three of four ROO movement sensors failed to work properly. Data from one ROO movement sensor showed that VIP infusion did not affect the ROO closure duration (1.52 ± 0.19 s) coinciding with the first phasic contraction of reticular biphasic contraction, comparing to that of saline infusion (1.49 ± 0.33 s), but duration of mainly regular ROO opening after the second phasic contraction of reticular EMG activity had slightly longer duration (3.85 ± 1.07 s vs 3.66 ± 0.86 s) in VIP infusion than that in saline infusion.

Effect of Temperatures

Data from exp.3, showed that the cold environmental temperature significantly stimulated EMG activity of the reticulum and omasum, but not of the ROO (Table 2-5). Reticular EMG frequency was significantly ($P < 0.05$) increased by 19.9% and quiescent duration of reticular EMG was significantly decreased by 23.7% in the cold environment, comparing to those in the warm environment. Active duration of reticular EMG was not influenced by environmental temperature. Although there was a 25.0% higher ROO EMG frequency and 43.9% shorter ROO EMG quiescent duration in the cold environment, these changes were not significant (Table 2-5). Active duration of the ROO was also not influenced by environmental temperature. Cold exposure induced a 60% increase ($p<0.001$) of frequency of omasal EMG bursts and a 57.9% decrease of quiescent duration ($P<0.001$) for omasal EMG, comparing to that in warm environment. Active duration of omasal EMG did not differ between cold and warm exposure.

Interaction of temperatures and VIP on EMG activity

During experiment three, there was significant interaction between the environmental temperature and VIP infusion on EMG activity of the reticulum and ROO (Table 2-6). VIP infusion significantly inhibited reticular EMG activity with lower ($p < 0.02$) frequency and longer ($P < 0.01$) quiescence in the warm environment. VIP infusion also significantly increased ($P < 0.05$) quiescent duration of ROO EMG activity in the warm environment.

There was no interaction of environmental temperatures and VIP infusion on omasal EMG activity.

Pressure change in gastrointestinal lumen

In experiment four, VIP modified gastrointestinal luminal pressure. Both amplitude and frequency of pressure at all sites were reduced by VIP, but there was no change in pressure duration (Figure 2-6). The majority of the pressure peaks were of relatively short duration. However, occasionally the observed periods of maximum pressure duration were plateau periods with high pressure tone accompanied by small amplitude pressure peaks. These plateau periods were observed relatively infrequently (at approximately hourly intervals) and sometimes lasted for 1-2 minutes. Although VIP did not significantly change the mean values of frequency, peak pressure duration and quiescence duration for 10 min of ruminal contractions, the maximum pressure period was significantly reduced by VIP in rumen ventral sac and proximal duodenum (Table 2-7). On the other hand, the minimum peak pressure duration ($p<0.06$) and quiescent duration ($P < 0.05$) in rumen and duodenum were increased by VIP. As well, similar results were shown in the ileum although these were not statistically significant except for increased ($P < 0.01$) minimal quiescent duration (Table 2-8). These results indicate an inhibitory role of VIP in the gastrointestinal tract.

D. Discussion

The general pattern of EMG activity in the reticulum, ROO and omasum in this study was similar to previous reports (Reid et al., 1988a,b; Okine and Mathison, 1996). The active duration for each reticular component of EMG activity was far shorter than data of Ali and Singleton (1974). Possible reasons for the differences may be due to animal breed, time after feeding and/or recording technique. We used a computerized recording system and data was analyzed by computing software (AcqKnowledge system) which could enlarge the chart to allow a very fine check on the timing of different parameters.

Besides regular activity of ROO EMG aligned with reticular EMG, there are up to two irregular EMG activities occurring between regular activities observed in present study,

which was similar to report (3-5 min^{-1}) by Reid et al. (1988b) in anaesthetized lambs. Comparing ROO EMG trace with the curve of the ROO movement sensor indicated that the ROO was relatively closed only during some high burst period of ROO EMG and the initial time of ROO closure started earlier than the onset of high ROO EMG burst. Some of ROO closures were not related to high bursts of ROO EMG activity. The time differential between the apparent onset of ROO EMG bursts and ROO closure might have resulted from the sampling rate of electronic signals at five readings per second. This rate might not have captured all of the EMG signals in present study. During low activity of ROO EMG, the ROO movement sensor readings did not appear to detect changes of ROO diameter. It is hard to tell if this is true or false because the sensitivity of the movement sensor may not have been enough to detect small changes in ROO diameter. Because no particular EMG signal was related to the ROO opening signals, the detected ROO opening in present study may have been mainly caused by the increased pressure inside the lumen due to digesta passage. The regular opening which followed after ROO EMG peak or the second peak of reticular biphasic activity suggests that there is a large volume of digesta from the reticulorumen passing through the ROO at this time. Also at this time the omasum is in relative relaxation (low EMG activity). Balch et al. (1951) reported that the lowest pressure in the omasum occurs 3 s after the second biphasic reticular contraction. This would be coincident with the wider opening of the ROO observed in the present study. Our results further confirm the endoscopic observation in sheep by Ruckebusch (1970) that the ROO closure takes place whenever the reticulum is contracted. This closure may be due to the distortion of the musculature of the oesophageal groove, which surrounds this orifice, during contraction of the reticulum. Newhook and Titchen (1972) speculated that ROO opening might be achieved by either inhibition of spontaneous contractions of the ROO by VIP or by activity of muscle near or in the ROO. The current data indicates that the ROO may be lightly relaxed at most times during the quiescence of the reticulum with one or more wider opening events. ROO opening during the second phasic contraction of the reticulum in cattle (Stevens et al., 1960; Balch and Campling, 1962) and dairy cows (Balch et al., 1951) were nerve found in the present study with sheep. The motility of the forestomach (Ruckebush, 1988) and digesta

kinetics (Colucci et al., 1990) between bovine and ovine is considerably different, so that caution has to be taken when using results from one species to extrapolate into the other.

The large variation of omasal EMG activity on active duration and quiescence in the present study, is consistent with several previous reports (Balch et al., 1951; Ohga et al., 1965; Ruckebusch, 1970; Okine et al., 1995), and suggest that regulation of omasal motility is complex.

The transient inhibitory effect of VIP on reticular EMG activity in present study in exp.1 and 3 is similar to the finding in anaesthetized lambs that infusion of VIP at 3 nmol per min for 10 min had no effect on the mean frequency of the biphasic reticular EMG bursts compared with the average number of bursts in the 10 min periods before or after the infusion (Reid et al., 1988b). A significant effect of VIP on reticular EMG activity in exp.2 was consistent with the response reported by Reid et al. (1988b) to 1-2 nmol VIP dose level. The general tendency of VIP to inhibit reticular EMG activity, especially during 4 to 9 min of the 10 min infusion period, supports results of a previous study where infusion of VIP depressed frequency of reticular EMG activity by 15% as comparing to saline infusion (Okine and Mathison, 1996).

Vasoactive intestinal polypeptide depressed the frequency of ROO EMG activity temporarily during 3-8 min of infusion period (Figure 2-5). This agrees, in part, with previous reports (Reid et al., 1988b; Okine et al., 1995; Okine and Mathison, 1996) in which there was a complete cessation of activity of low amplitude EMG bursts and a diminution of EMG activity of high amplitude bursts of the ROO in the conscious meal-fed sheep. Inhibition of ROO EMG activity during infusion of exogenous VIP (3 nmol min^{-1}) was also found in anaesthetized lambs and the conscious milk-fed lamb (Reid et al., 1988a,b), but there was no consistent effect of VIP at a lower dosage (1.5 nmol min^{-1}) rate. In the present study, VIP inhibition may have been partially masked by the initial stimulation noted for both saline and VIP at the start of infusion. Reid et al. (1988b) also found that there was an increase in frequency and magnitude of the ROO EMG for up to 120 s during commencement of the VIP infusions. Moreover, Reid et al. (1988a) describe their data in the following words: "the pattern of pressure fluctuations recorded from the ROO of lambs with

one or both vagus nerves intact was not usually as regular as that observed when both vagi were cut". They demonstrated that there are both excitatory and inhibitory effects of the vagus on the ROO such that vagus stimulation at lower frequencies (<20 Hz) created excitation of activity of the ROO whereas higher frequencies (50-70 Hz) caused inhibition. Okine and Mathison (1996) also found that the ROO activity was never completely abolished during VIP infusion and low amplitude bursts of EMG activity of the ROO reappeared 3 to 4 min after VIP infusion was terminated. As well, at higher doses of 8-10 nmol VIP per min the inactivity of ROO EMG developed within 30 s and was continued for only 30-120 s, after which the activity of the ROO returned (Reid et al., 1988b). These indicate that the effect of VIP on ROO activity is variable and the functional effect of VIP as a neurotransmitter in the inhibitory response of the ROO is not fully understood. The increase of duration of ROO opening during VIP infusion suggests that the inhibitory effect of VIP on ROO myoelectric activity might facilitate outflow of digesta from reticulorumen to the omasum because of relative delimitation of its narrowness. Inhibition of contractions of the ROO are believed to occur in the lamb when it sucks, permitting passage of milk caudally to the abomasum (Reid and Titchen, 1988).

The different effects of VIP in different part of the forestomach may indicate potential variations in receptor density and subtypes (Molinero et al., 1985; Wathuta, 1986) in different regions. Alternatively, they may reflect interactions with other regulators of EMG activity, such as galanin and neuropeptide Y (Fox-Threlkld et al., 1991; Harada et al., 1992).

The thermal environment significantly influenced frequency and quiescent duration of reticular EMG (Table 2-5), consistent with the previous report by Westra and Christopherson (1976). The reduced quiescence duration is an likely inevitable consequence of increased frequency. The latter authors found a significant increase in the frequency of reticular contraction in closely shorn sheep subjected to cold treatment. However, no significant change of frequency of reticular contraction was observed in another study with unshorn lambs under chronic cold exposure (Christensen et al., 1990). The closely shorn sheep would have been more responsive to cold because of reduced insulation. This reason may help to explain contrary result from closely shorn sheep in present study with Okine et

al. (1995) who suggested that environmental temperatures between 0 and 20 °C do not have a marked influence on reticular EMG activities. The relatively constant active duration of reticular EMG activity in the present study is also not consistent with the finding of Miaron and Christopherson (1992) that the duration of reticular contractions at rest was significantly reduced at -10 °C in steers, compared to duration at both 10 and 28 °C.

The significant interaction of VIP infusion treatment and thermal environment on frequency and quiescence of reticular EMG activity indicates that modification of rhythm of activivity is a major factor to influence reticular function. At the same time, the smaller effect of VIP on reticular EMG in the cold compared to the warm environment (Table 2-6) suggests that cold temperature may have masked the effect of VIP on reticular contraction.

Effect of environmental temperatures on ROO EMG activity in the present study (Table 2-6) was not consistent with the finding of Okine et al. (1995) that cold acclimated sheep had a 28% increase in the duration of ROO quiescence compared to warm acclimated sheep. The reason for these differences is not clear. Significant interaction of environmental temperatures and infusion treatments on quiescence of ROO EMG activity also indicated that stimulation of cold exposure masked VIP effects.

Omasal EMG activity was more responsive to effects of thermal environment. Changes of frequency (60%) and quiescence (137%) of EMG in response to the cold exposure were much bigger than those of the reticulum and ROO (Table 2-5). This evidence may suggest that the omasum could play a more powerful role on in controlling digesta passage in the cold than the reticulum and ROO.

The significant difference in the frequency and quiescence of EMG activity of the omasum under different thermal environments suggests that 1 ± 1.5 °C was below the lower critical temperature of shorn sheep. It may also suggests that the omasum is an important part to regulate digesta flow in a cold environment. The fleece regrowth during 3-7 weeks of acclimatization and trial periods apparently could not ameliorate the effects of the cold environment on myoelectric activities in the sheep.

Increases in passage rate of digesta in the cold have been associated with increases in frequency of reticular contractions (Westra and Christopherson, 1976; Gonyou et al.,

1979; Kennedy et al., 1986) although Mathison et al. (1995) suggested that rumen outflow is not closely related to reticular frequency. There might be a change in amount of digesta outflow per contraction of the reticulum as reported by Miaron and Christopherson (1992). Increased reticular contraction may move large particles in the reticular digesta so that the chance of obstruction is reduced, and also may increase the total time of a positive pressure differential between the reticulum and omasum. In such a situation, the outflow of fine particles and liquid of the ruminoreticular digesta would be enhanced subsequently if the ROO were relaxed and not obstructed by the lips of the reticular groove. However, relaxation of the ROO by VIP was not accompanied by an increased frequency of reticular EMG activity in the present experiments. The increased opening of the ROO under VIP in the warm environment is an indication of its potential (dynamic role) in regulating digesta outflow from reticulorumen to the omasum. The higher pressure of ruminal contraction after the reticular contraction is a possible dynamic force to provide a positive pressure difference through the ROO when the omasum is in relaxation, thereby enhancing digesta outflow from the reticulorumen.

The change of ruminal pressure mainly reflects both primary and secondary contractions of rumen in the rumen ventral sac (Peruzzo-de-Naville et al., 1987) since most of the time, waves of the secondary contractions are mixed with primary contractions. The secondary contraction functions mainly in eructation of ruminal gas. Pressure changes in the ventral sac of the rumen showed that VIP depressed ruminal contractions in amplitude with less effect on pressure frequency and duration (Figure 2-6 and Table 2-7). The pressure pattern of the small intestine in present study did not have so much variation as reported by Ruckebusch (1970). In this study, observed long high-pressure period (refer as maximum pressure duration) is likely representative in a mixed form of digesta flow and peristaltic wave involved in propulsion of chyme (Ruckebusch, 1970). Effects of VIP on the duodenal pressure pattern indicated its inhibitory role, but it is hard to tell how these pressure peaks related to intestinal contraction and digesta flows. In the ileum the motility is quite different and tonic changes appear to occur with the propulsive contractions as reported previously by Ruckebusch (1970). The inhibitory effect of VIP on the terminal ileum contraction further

suggests a broad role of VIP on regulation of gastrointestinal motility and a possible clue that VIP infusion via gastric artery catheter at 1.5 nmol ml^{-1} min^{-1} may have had potential effects mediated by the central and peripheral regulation.

In conclusion, the data from this study shows that: 1) exogenous VIP transiently inhibited EMG activities of the reticulum, ROO and omasum mainly by modifying rhythm of myoelectrical activity to increase quiescence, 2) gastrointestinal luminal pressure patterns were also transiently influenced by VIP with an increase of quiescent time in the warm environment, 3) cold exposure of sheep stimulated a higher motility in the reticulum and omasum, 4) diameter change of the ROO was not closely related to its EMG activity, 5) VIP increased opening duration of the ROO and extended the quiescence duration of the ROO EMG activity in a warm environment.

Figure 2-1. The movement sensor loop design. The circumference of the loop is 4.5 cm.

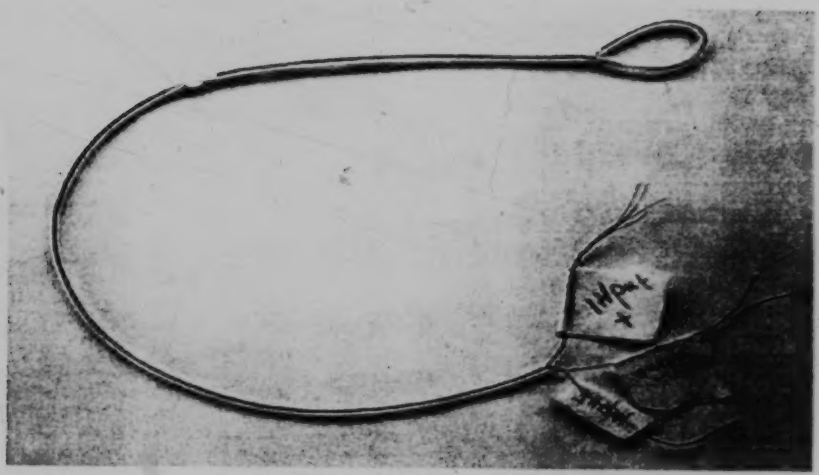
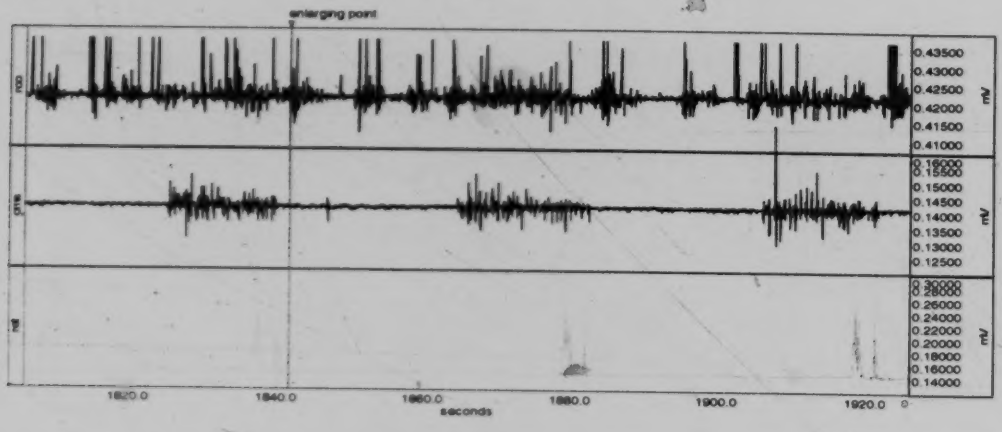


Figure 2-2. Electromyographic (EMG) activity of the reticulum (ret), reticulo-omasal orifice (ROO) and omasum (oma) (exp.1). A: Three active periods in all three channels occur in 2 min. The relative quiescence (no activity) of ROO myoeletric activity is very short and happens at the quiescent period of reticular and omasal electric activities. The active duration (duration from initial point of spike to the end of spike) of reticular EMG is the shortest, ROO EMG the longest, and omasal EMG intermediate. B: enlarged part of A shows that the EMG of the omasum ends and ROO EMG arrests prior to the secondary contraction of reticular EMG.

A:



B:

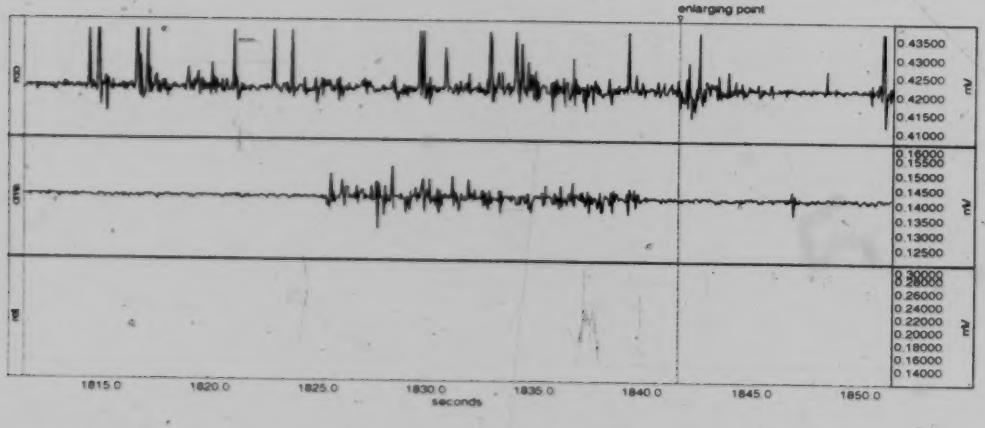


Table 2-1. Electromyographic (EMG) activity averaged for 10 min prior to and 20 min after feeding at the reticulum, reticulo-omasal orifice (ROO) (exp.1-3).

Items	Reticular EMG		ROO EMG		Omasal EMG	
	Prior to	After	Prior to	After	Prior to	After
Frequency[Z]	1.33	2.47	.1.23	2.47	0.94	2.73
SE	0.04	0.18	0.11	0.20	0.03	0.26
Active duration (s)[Y]	8.5	6.3	27.1	16.3	18.7	14.1
SE	0.1	0.1	1.7	1.4	3.5	2.2
Quiescence (s)[X]	36.5	18.1	21.6	8.0	45.1	7.9
SE	0.7	0.8	2.7	1.3	3.3	1.0

Note: Z - unit of frequency is cycles per minute.

Y - duration from the initial point of spike to the end of spike.

X - duration of no activity.

Figure 2-3. Relationship of reticulo-omasal orifice (ROO) opening and reticular EMG activity (ret) in the warm environment in experiment 1. The downward excursion of signals from the movement sensor (moving sensor) means decrease of ROO diameter (closure) and the upward excursion means increase of ROO diameter (opening).

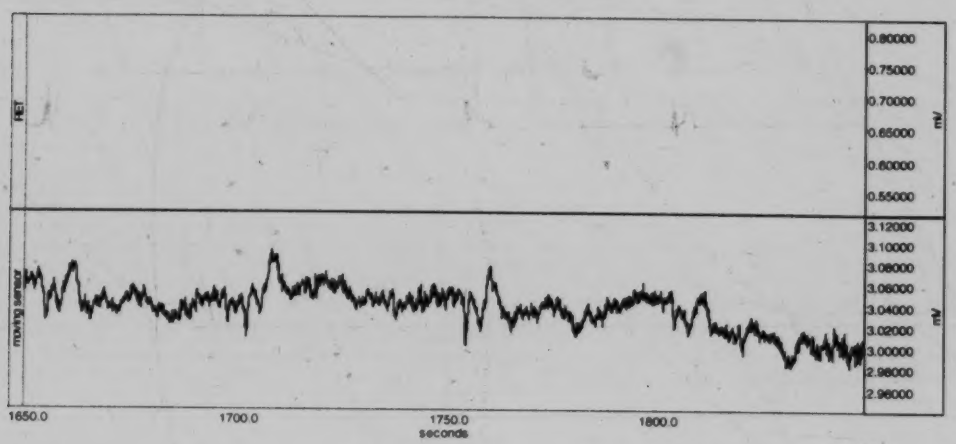


Figure 2-4. Electromyographic (EMG) activity in the forestomach. The channels from top to bottom are reticulo-omasal orifice (ROO), omasal (oma) and reticular (ret) EMG signals. Horizontal scale unit is minute and vertical scale unit is millivolt (mV). VIP infusion inhibited the EMG activity of the reticulum, ROO and omasum.

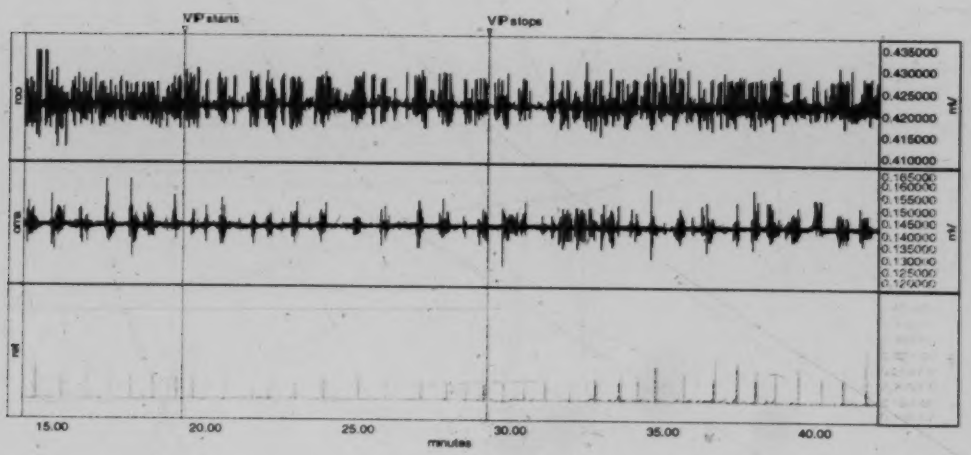
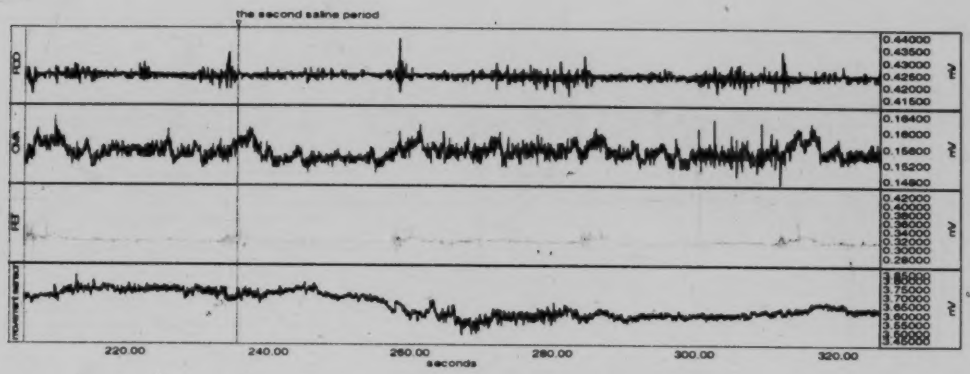


Figure 2-5. Electromyographic (EMG) and movement activities in the forestomach during saline and VIP infusion period in exp.1. A and B charts come from the same animal, and are selected from 120 s records of saline (A) and VIP (B) infusion periods at the same duration of the second infusion. In each chart, channels from top to bottom are reticulo-omasal orifice (ROO), omasal (oma) and reticular EMGs (ret) and the ROO movement sensor signal. Horizontal scale unit is second and vertical scale unit is millivolt (mV).

A:



B:

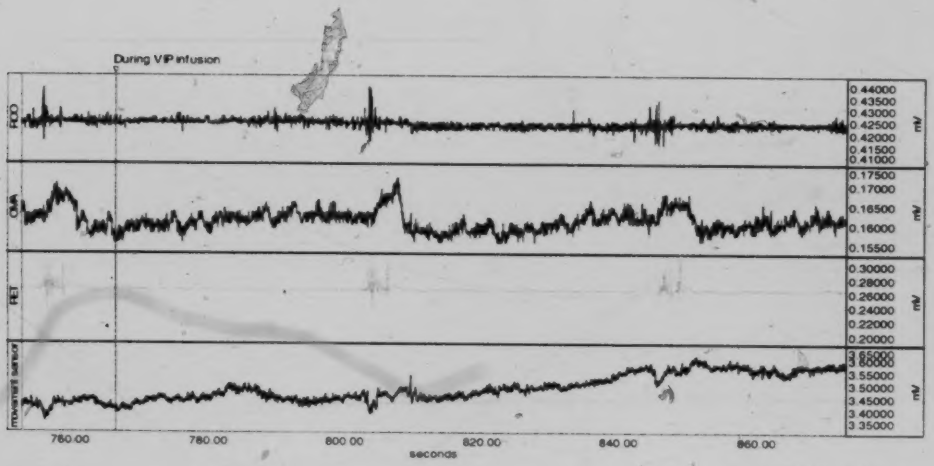


Table 2-2. Effects of VIP and saline infusion on EMG activity of the reticulum, reticulo-omasal orifice (ROO) and omasum in exp.1.

item	VIP*	Saline*	P
Reticular EMG			
Frequency (cycle min^{-1})	1.793 ± 0.092	1.979 ± 0.098	0.183
Active duration (s)	4.373 ± 0.428	5.301 ± 0.457	0.155
Quiescence (s)	29.719 ± 1.432	26.823 ± 1.528	0.182
ROO EMG			
Frequency (cycle min^{-1})	2.651 ± 0.318	2.969 ± 0.285	0.087
Active duration (s)	11.422 ± 1.842	8.356 ± 1.132	0.095
Quiescence (s)	14.133 ± 3.069	13.622 ± 2.469	0.091
Omasal EMG			
Frequency (cycle min^{-1})	1.576 ± 0.069	1.758 ± 0.075	0.089
Active duration (s)	20.304 ± 1.673	20.688 ± 1.811	0.878
Quiescence (s)	20.628 ± 1.232	15.602 ± 1.333	0.012

Note: * The value of each parameter was expressed by least square mean (Lsmeans) ± SE. The number of observations was 12 for each parameter.

Table 2-3. Effects of VIP and saline infusion on EMG activity of the reticulum, reticulo-omasal orifice (ROO) and omasum in exp.2.

item	VIP*	Saline*	P
Reticular EMG			
Frequency (cycle min^{-1})	1.473 ± 0.038	1.617 ± 0.041	0.021
Active duration (s)	3.851 ± 0.297	4.029 ± 0.315	0.687
Quiescence (s)	39.259 ± 1.108	35.318 ± 1.175	0.028
ROO EMG			
Frequency (cycle min^{-1})	1.799 ± 0.078	2.014 ± 0.144	0.162
Active duration (s)	16.429 ± 1.399	17.949 ± 2.142	0.326
Quiescence (s)	17.375 ± 1.223	13.017 ± 2.125	0.172
Omasal EMG			
Frequency (cycle min^{-1})	2.195 ± 0.231	2.325 ± 0.229	0.358
Active duration (s)	9.363 ± 0.225	17.605 ± 1.692	0.011
Quiescence (s)	17.998 ± 2.798	9.220 ± 2.207	0.018

Note: * The value of each parameter was expressed by lsmean ± SE. The number of observations was 12 for each parameter.

Table 2-4. Effects of VIP and saline infusion on EMG activity of the reticulum, reticulo-omasal orifice (ROO) and omasum in exp.3.

item	VIP*	Saline*	P
Reticular EMG			
Frequency (cycle min^{-1})	1.324 ± 0.071	1.429 ± 0.058	0.135
Active duration (s)	4.462 ± 0.312	4.233 ± 0.351	0.320
Quiescence (s)	47.866 ± 3.501	40.923 ± 2.107	0.102
ROO EMG			
Frequency (cycle min^{-1})	2.791 ± 0.281	2.904 ± 0.247	0.191
Active duration (s)	13.291 ± 2.084	15.155 ± 1.817	0.506
Quiescence (s)	11.454 ± 2.290	8.531 ± 1.308	0.278
Omasal EMG			
Frequency (cycle min^{-1})	1.955 ± 0.170	2.093 ± 0.199	0.603
Active duration (s)	15.860 ± 1.691	15.827 ± 2.006	0.990
Quiescence (s)	21.374 ± 2.689	17.234 ± 2.936	0.307

Note: * The value of each parameter was expressed by lsmean ± SE. The number of observations was 24 for each parameter.

Table 2-5. Effects of environmental temperatures on EMG activity of the reticulum, reticulo-omasal orifice (ROO) and omasum in exp.3.

item	Cold*	Warm*	P
Reticular EMG			
Frequency (cycle min^{-1})	1.482 ± 0.050	1.236 ± 0.076	0.012
Active duration (s)	4.669 ± 0.322	3.957 ± 0.305	0.119
Quiescence (s)	39.478 ± 1.892	51.708 ± 3.327	0.010
ROO EMG			
Frequency (cycle min^{-1})	3.043 ± 0.226	2.435 ± 0.282	0.110
Active duration (s)	14.102 ± 1.598	14.281 ± 2.760	0.956
Quiescence (s)	7.982 ± 0.727	10.238 ± 3.547	0.120
Omasal EMG			
Frequency (cycle min^{-1})	2.320 ± 0.152	1.447 ± 0.117	0.001
Active duration (s)	15.652 ± 1.450	16.203 ± 2.530	0.852
Quiescence (s)	13.235 ± 1.368	31.466 ± 2.769	0.001

Note: * The value of each parameter was expressed by lsmean ± SE. The number of observations was 24 for each parameter.

Table 2-6. Interaction of environmental temperatures and VIP infusion on EMG activity of the reticulum, reticulo-omasal orifice (ROO) and omasum in exp.3.

Item	Cold		Warm		Pooled	P
	VIP	Saline	VIP	Saline	SE	
Reticular EMG						
Frequency (cycle min^{-1})	1.478^{a}	1.487^{a}	1.170^{b}	1.345^{a}	0.08	0.02
Active duration (s)	5.245	4.083	3.669	4.476	0.43	0.08
Quiescence (s)	39.132^{b}	39.823^{b}	56.599^{a}	42.904^{b}	4.58	0.01
ROO EMG						
Frequency (cycle min^{-1})	2.984	3.109	2.310	2.536	0.29	0.32
Active duration (s)	15.001	13.480	9.018	18.170	3.59	0.74
Quiescence (s)	8.200^{b}	7.740^{b}	15.590^{a}	9.956^{b}	3.42	0.05
Omasal EMG						
Frequency (cycle min^{-1})	2.356^{a}	2.277^{a}	1.354^{b}	1.633^{b}	0.19	0.01
Active duration (s)	13.924	17.726	18.764	11.080	3.11	0.06
Quiescence (s)	14.104^{b}	12.191^{b}	32.278^{a}	29.843^{a}	2.84	0.01

Note: * The value of each parameter was expressed by lsmean ± SE. The number of observations was 12 for each parameter.

a, b means value with different superscript in the same row is significant different at level of P value.

Figure 2-6. Pressure pattern recorded in an individual sheep prior to and during VIP infusion in the rumen, proximal duodenum and the terminal ileum. The channels from top to bottom are the ventral rumen sac, proximal duodenal and terminal ileum (exp.4).

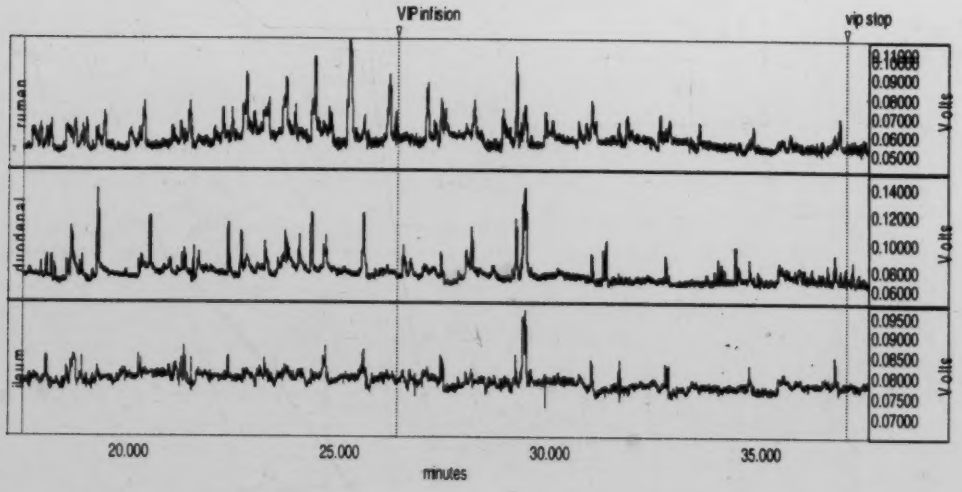


Table 2-7. Mean values for luminal pressure peaks in the rumen and duodenum (exp.4).

Item	VIP	Saline	Significance
Rumen [z]			
Frequency (peaks/min)	1.21 ± 0.13	1.33 ± 0.09	0.431
Peak pressure duration (s)	19.39 ± 2.24	17.91 ± 2.46	0.663
Max (s)	32.23 ± 2.41	66.23 ± 11.86	0.014
Min (s)	8.90 ± 2.15	4.38 ± 0.68	0.064
Quiescence (s)	36.33 ± 4.08	26.77 ± 3.74	0.106
Max (s)	72.38 ± 15.01	78.58 ± 15.11	0.775
Min (s)	19.00 ± 3.12	5.68 ± 2.04	0.003
Duodenum [y]			
Frequency (peaks/min)	1.29 ± 0.22	1.31 ± 0.09	0.916
Peak pressure duration (s)	10.39 ± 2.54	15.69 ± 2.27	0.143
Max (s)	29.68 ± 10.86	129.95 ± 26.05	0.003
Min (s)	2.83 ± 0.27	1.90 ± 0.19	0.015
Quiescence (s)	52.74 ± 15.92	32.31 ± 2.49	0.225
Max (s)	131.60 ± 35.43	143.25 ± 35.12	0.819
Min (s)	17.23 ± 6.73	3.05 ± 0.69	0.055

Notes: z the number of observation was 15 for each parameter.

y the number of observation was 12 for each parameter.

Table 2-8. Mean values for luminal pressure peaks in the ileum [z] (exp.4).

Item	VIP	Saline	Significance
Frequency (peaks/min)	0.89 ± 0.12	1.14 ± 0.08	0.113
Peak pressure duration (s)	13.58 ± 2.86	16.04 ± 2.20	0.508
Max (s)	36.17 ± 8.57	127.05 ± 51.41	0.107
Min (s)	3.20 ± 0.70	1.91 ± 0.32	0.120
Quiescence (s)	61.32 ± 10.83	38.72 ± 4.50	0.078
Max (s)	141.60 ± 23.16	158.46 ± 20.67	0.597
Min (s)	8.97 ± 1.88	3.09 ± 0.51	0.011

Notes: z the number of observation was 15 for each parameter.

E. Literature Cited

Ali, T. M., and A. G. Singleton. 1974. Pressure changes in the reticulum of sheep. J. Physiol. 240: 44p-46p.

Balch, C. C., A. Kelly, and G. Heim. 1951. Factors affecting the utilization of food by dairy cows. 4. The action of the reticulo-omasal orifice. Br. J. Nutr. 5: 207-216.

Balch, C. C., and R. C. Campling. 1962. Regulation of valuntary food intake in ruminants. Nutritional Abstr. Rev. 32: 669-686.

CCAC. 1993. Guide to the Care and Use of Experimental Animals. Vol. 1. Canadian Council on Animal Care, Ottawa, ON.

Christensen, R. A., R. J. Christopherson, and J. J. Kennelly. 1990. Effect of somatostatin and chronic cold exposure on hormonal and metabolite concentration, metabolic rate, thermoregulation, and gut motility in sheep. Can. J. Anim. Sci. 70: 1073-1083.

Christopherson, R. J. 1984. Effects of cold environment on digesta and feed intake of ruminants. Farming for the Future, Final Report of project: 81-0022 (internal reference)

Christopherson, R. J. 1989. In Proceedings of the First International Symposium on Agricultural Technique for Cold Regions, Obihiro, pp. 81-89.

Cowan, R.T., R. J. Moss, and D. V. Kerr. 1993. Northern dairy feedbase 2001:2. Summer feeding system. Tropical Grasslands. 27(3): 150-161.

Denac, M., C. Oertle, G. Kumin, E. Eggenberger, and E. Scharrer. 1990. Relaxation of muscle strips from the reticular groove and reticulo-omasal orifice by vasoactive intestinal peptide. J. Vet. Med., Series A 37:425-429.

Denac, M., J. Marti, and E. Scharrer. 1987. Relaxation of ruminal smooth muscle by vasoactive intestinal polypeptide. Zentralblatt Fur Veterinamedizin -Reihe A. 34(4): 317-320.

Fox-Threlkeld, J. E., H. Manaka, Y. Manaka, S. Cipris, and E. E. Daniel. 1991. Stimulation of circular muscle motility of the isolated perfused canine ileum: relationship to VIP output. Peptides. 12(5): 1039-45.

Gonyou, H. W., R. J. Christopherson, and B. A. Young. 1979. Effects of cold temperature

and winter condition on some aspects of behaviour of feedlot cattle. Appl. Anim. Ethol. 5: 113-124.

Harada, Y., M. Okubo, K. Yaga, T. Kaneko, and K. Kaku. 1992. Neuropeptide Y inhibits beta-adrenergic agonist- and vasoactive intestinal peptide-induced cyclic AMP accumulation in rat pinealocytes through pertussis toxin-sensitive G protein. J. Neurochem. 59(6): 2178-83.

Kennedy, P. M. 1985. Influence of cold exposure on digestion of organic matter, rate of passage of digesta in the gastrointestinal tract, and feeding and rumination behaviour in sheep given four forage diets in chopped or ground and pelleted form. Br. J. Nutr. 53: 159-173.

Kennedy, P. M., R. J. Christopherson, and L. P. Milligan. 1986. Digestive response to cold. Pages 285-306 *in* L. P. Milligan, W. L. Grovum and A. Dobson, eds. Control of digestion and metabolism in ruminants. Prentice-Hall, Englewood Cliffs, NJ.

Kennedy, P.M., and L. P. Milligan. 1978. Effect of cold exposure on digestion, microbial synthesis and nitrogen transformation in sheep. Br. J. Nutr. 39: 105-117.

Kingma, Y. J., K. L. Bowes, M. S. K. Kocylowski, and J. Szmidt. 1980. Inductive displacement gauge. American Journal of Physiology. 239 (Gastrointest. Liver Physiol. 2): G128-G132.

Luts, L., and F. Sandler. 1994. Peptide-containing nerve fibers in the parathyroid glands of different species. Regulatory Peptides. 50(2): 147-158.

Mathison, G. W., E. K. Okine, A. S. Vaage, M. Kaske, and L. P. Milligan. 1995. Current understanding of contribution of the propulsive activities in the forestomach to flow of digesta. In: W. V. Engelhardt, S. Leonhard-Marek, G. Breves, and D. Giesecke (Ed.) Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. pp 23-41. Ferdinand Enke Verlag Stuttgard, Germany.

Miaron, J. O. O., and R. J. Christopherson. 1992. Effect of prolonged thermal exposure on heat production, reticular motility, rumen-fluid and -particulate passage-rate constants, and apparent digestibility in steers. Can. J. Anim. Sci. 72: 809-819.

Molinero, P., J. R. Calvo. R. Goberna, and J. M. Guerrero. 1985. The interaction of

vasoactive intestinal pep ide (VIP) with isolated bovine thyroid plasma membranes. Biochemical & Biophysical Research Communications. 128(3): 1336-41.

Newhook, J. C., and D. A. Titchen. 1972. Effect of stimulation of efferent fibers of the vagus on the reticulo-omasal orifice of the sheep. J. Physiol. 222: 407-418.

Ohga, A., Y. Ota, and Y. Nakasato. 1965. The movement of the stomach of sheep with special reference to the omasal movement. Jap. J. Vet. Sci. 27: 151-160.

Okine, E, K., and G. W. Mathison. 1996. Effects of vasoactive intestinal polypeptide on electromyographic activity of the reticulo-omasal orifice in sheep at two levels of intake. J. Anim. Sci. 74(7): 1681-1688.

Okine, E. K., J. O. O. Miaron, S. Cosgrove, and R. J. Christopherson. 1995. Vasoactive intestinal polypeptide inhibits electromyographic activity of the reticulo-omasal orifice in meal-fed sheep in warm and cold environments, Can. J. Anim. Sci. 75: 333-339.

Peruzzo-de-Naville, L. E., H. W. Jr. Colvin, and R. C. Backus. 1987. The primary-secondatry rumen contraction and gas expulsion in sheep (Ovis aries). Comp. Biochem. Physiol. A Comp. Physiol. 87(4): 993-1002.

Reid, A.M., and D. A. Titchen. 1984. Effects of vasoactive intestinal polypeptide on the reticulo-omasal orifice of the lamb. Canadian Journal of Animal Science. 64 (Suppl.): 91-92.

Reid, A.M., and D. A. Titchen. 1988. Gastric electromyographic activity in the milk-fed lamb. Journal of Physiology. 396: 25-39

Reid, A.M., A. Shulkes, and D. A. Titchen. 1988a. Effects of the vagus nerves on gastric mobility and release of vasoactive intestinal peptide in the anaethetized lamb. J. Physiol. 396:11-24.

Reid, A.M., A. Shulkes, and D. A. Titchen. 1988b. The vasoactive intestinal polypeptides on gastric motility in the lamb. J. Physiol. 396:41-45.

Ruckebusch, Y. 1970. The electrical activity of the digestive tract of the sheep as an indication of the mechanical events in various regions. J. Physiol. 210: 857-882.

Ruckebush, Y. 1988. Motility of the gastrointestinal tract. In: Church D. C. Ed. The ruminant

animal. Englewood Cliffs, NJ.

Said, S. I. 1984. Vasoactive intestinal polypeptide (VIP): Current status. Peptides. 5:143-150.

SAS. 1996. Version 6.12, SAS Institute, Inc. Cary, NC.

Stevens, C. E., A. F. Seller, and F. A. Spurrel. 1960. Function of the bovine omasum in digesta transfer. Am. J. Physiol. 198: 449-455.

Toullec, R., J. A. Chavialle, P. Guilloteau, and C. Bernard. 1992. Early-patterns of plasma gut regulatory peptide levels in calves. Effect of age, weaning and feeding. Comp. Biochem. Physiol. 102A: 203-209.

Wathuta, E. M. 1986. The distribution of vasoactive intestinal polypeptide-like, substance P-like and bombesin-like immunoreactivity in the digestive system of the sheep. Q. J. Exp. Physiol. 71: 615.

Westra, R., and R. J. Christopherson. 1976. Effects of cold on digestibility, retention time of digesta, reticulum motility and thyroid hormones in sheep. Can. J. Anim. Sci. 56: 699-708.

Yamamoto, Y., N. Kitamura, J. Yamada, and T. Yamashita. 1994. Immunohistochemical study of the distributions of the peptide- and catecholamine-containing nerves in the omasum of the sheep. Acta Anatomica. 149(2): 104-10.

CHAPTER THREE

EFFECT OF VIP INFUSION ON FEED INTAKE, DIGESTA FLOW AND NUTRIENT DIGESTION IN SHEEP WITH TIME LIMITED ACCESS TO FEED

A. Introduction

Vasoactive intestinal polypeptide (VIP), as a neurotransmitter, has been demonstrated to influence gastrointestinal (GIT) activity by both direct effects on the gut tissue (Lam, 1991) and by indirect effects through modification of central nervous system (CNS) - mediated neuroendocrine reflexes (Seid, 1984; Reid et al., 1988a; Luts and Sundler, 1994). There is a wide distribution of VIP receptors in the esophagus, antrum, fundus, duodenum, jejunum, ileum and colon. Indeed there is a particularly high density of VIP receptors in the region of the reticulo-omasal orifice (Wathuta, 1986; Zdon et al., 1988) and in the inner muscle layer of the omasal wall (Yamamoto et al., 1994) of ruminants.

Central infusion of VIP stimulates the release of growth hormone, prolactin and adrenocorticotropic hormone (Lam, 1991), and stimulates gastric acid secretion in a dose-, time- and vagus-dependent manner (Hernandez et al., 1987). Central infusion of VIP also produces a significant inhibition of alanine and water absorption in the jejunum (Saade et al., 1995).

Local VIP infusion directly affects digestive gland secretion. Increased pancreatic flow rate (Rodriguez-Lopez et al., 1995; Kiela et al. 1996), protein and trypsin output in the pancreatic juice (Kiela et al. 1996) and bicarbonate output have been observed (Rodriguez-Lopez et al., 1995) in response to local infusion of VIP. Vasoactive intestinal polypeptide infusion also stimulates gastric secretion (Nassar et al., 1995), duodenal bicarbonate and chloride secretion, jejunal chloride secretion, and bicarbonate secretion in the ileum in the rat (Chikhlassa et al., 1992), and jejunal secretion of sodium, potassium, bicarbonate and water in sheep (Hyun et al., 1995). In addition, acetylcholine-mediated secretions of fluid and protein from rat submandibular glands were enhanced by intravenous injection of VIP (Iwabuchi and Masuhara, 1995). Moreover, suppressed chloride absorption in the ileum

(Chikhlssa et al., 1992) and water absorption (Nassar et al., 1995) were reported in response to VIP treatment. Vasoactive intestinal polypeptide significantly inhibited the intestinal absorption of [^{3}H]-leucine by 59.1% (Chen et al., 1987) and alanine by 60% (Nassar et al., 1995). These data indicate that exogenous VIP influences the gastrointestinal digestive juice, enzyme output and nutrient absorption.

VIP induces a concentration-dependent reduction of acetylcholine-induced isotonic contraction of muscle strips (Denac et al., 1987). Denac et al. (1987) reported that VIP was more effective in the muscle strips from the calves than those from adult cattle although Toullec et al., (1992) did not find any effect of age, weaning and feeding on plasma VIP. The circular muscle strips from the reticular groove, in vitro, were more sensitive to VIP than the longitudinal muscle strips. Both circular and longitudinal muscle strips from the omasal canal of the calf were relaxed by VIP (Denac et al., 1990). In addition, VIP produces cessation (quiescence) of electromyographic (EMG) activity of the reticulo-omasal orifice (ROO) in conscious and anaethetized lambs (Reid and Titchen, 1988; Okine et al., 1995; 1996). The extended quiescence of the ROO may increase the opportunity for passage of reticuloruminal digesta and, as a result, VIP may facilitate digesta passage through the ROO from the reticulorumen to the omasum by relaxing the ROO.

On the basis of available data about the effects of VIP on gastrointestinal secretion and absorption, gastrointestinal motility and especially the effective relaxation of the ROO, we hypothesized that exogenous VIP will stimulate feed intake, and alter digesta flow and nutrient digestion in ruminants.

The objectives of these studies were to investigate whether VIP infusion through the gastric artery would stimulate feed intake and digesta flow through the gastrointestinal tract, and influence nutrient digestibility.

B. Experimental Procedures

Animal Trial Procedures

Nine sheep ranging from 30-50 kg body weight were used in two consecutive experiments. Four sheep in experiment 1 and five sheep in experiment 2 were housed in floor pens during the acclimation and intervals of treatment periods and in metabolic crates during

infusion treatments in a thermoneutral environment (21 ± 2 °C). In Exp.1, the sheep were allowed free access to pelleted alfalfa (estimated ME 9.4 MJ kg^{-1}, CP 13.4% of DM) diet for 8 h per day. In Exp.2, sheep were fed an alfalfa pellet diet with a higher protein content (estimated ME 10 MJ kg^{-1}, CP 20.63 % of DM). All sheep were allowed free access to water and salt blocks.

In **Exp.1**, Sheep were surgically fistulated with ruminal and "T" type duodenal cannulae at proximal duodenum three weeks before the experiment started. Animals were also fitted with a left gastric artery polypropylene catheter (I.D. 0.58 and O.D. 0.96 mm) of about 100 cm in length. After surgery, sheep were given antibiotic therapy with long active Liquamycin (1 ml 20^{-1} kg of BW) for three days. Animals were cared for according to the guidelines of CCAC (1993) and given one week for recovery and two further weeks for familiarising with all equipment prior to commencement of any experimental data recording. Fresh drinking water and feed were provided at 0830am every day. Water consumption and feed intake were determined from the difference of supply and residue after 24 h for water and 8 h for feed every day during treatment periods.

During the treatment periods, VIP (Sigma Chemical Co., product #V3628, ST., Louis, MO) (1 nmol per min) or physiological saline solution, as a control, were infused with a pump (Ismatec MT-13SA, Zurich, Switzerland) through the gastric artery catheter. The infusion was given continuously for 10 min per hour for 3 h per day for 4 consecutive days and the first infusion started at 2 min before feeding. Before infusion treatments, all the ends of the tubing infusion lines were sterilized by 75% alcohol and then washed by saline. The infusion lines were sterilized by pumping 75% alcohol for 4 min and washed by pumping saline for 6 min. All infusion solutions were stocked in the covering bottles during treatments.

In **Exp.2**, Sheep were surgically fistulated with ruminal and "T" type duodenal and terminal ileal cannulae (Kelly et al., 1989). Sheep were also fitted with a gastric-artery catheter (I.D. 0.58 and O.D. 0.96 mm) of about 100 cm length. After surgery sheep were given antibiotic therapy with long active Liquamycin (1 ml 20^{-1} kg of BW) for three days. Animals were cared for according to the guidelines of CCAC (1993) and given one week for recovery and two weeks for familiarising with all equipment prior to commencement of any

experimental data recording. Fresh drinking water and feed were provided at 0830am every day. Water consumption and feed intake were determined from the difference of supply and residue after 24 h for water and 8 h for feed every day during treatment periods.

During the treatment periods, VIP (Purity 97.9%, Peptide Institute, University of Alberta, Edmonton, AB) (1.5 nmol per min) or physiological saline solution, as a control, were infused with a pump (Ismatec MT-13SA, Zurich, Switzerland) through the gastric artery catheter. The infusion was given continuously for 10 min each 30 min for 3 hours per day for 7 consecutive days starting at 2 min before feeding.

Marker preparation, administration and sampling

Chromium (Cr)-mordanted fibre prepared from timothy hay, which was ground to pass through a 1 mm screen, was used as a particulate marker to estimate DM duodenal passage. The fibre for mordanting was initially washed with ordinary detergent, rinsed thoroughly, and dried at 100 °C. The fiber was then soaked in potassium dichromate solution, covered and incubated at 70 °C for 48 h. The material was washed thoroughly and soaked for 10 h in 5% ascorbic acid. Lastly, the fiber was washed thoroughly until the rinse water was free of soluble green matter and dried at 70 °C for 48 h (Uden et al., 1980). The fluid passage rate was determined by use of Co-EDTA, prepared as outlined by Uden et al (1980).

In Exp.1, Cr-mordanted fiber and Co-EDTA were administered three times each day for 5 consecutive days from one day before the start of treatment in each period to the last day of treatment. In Exp.2, Cr-mordanted fiber and Co-EDTA were administrated during the last 6 days of the seven treatment days. Markers were given through the rumen cannula. Each of the three doses per day consisted of 6 g Cr-mordanted fibre and 1 g Co-EDTA.

Digesta samples were collected during the last 2 days of each treatment period. Approximately 50 ml of duodenal digesta and, in Exp.2, 20-30 ml ileal digesta samples were collected at 6 h intervals. The volume and weight of each of digesta sample were recorded. Total faeces were also collected during last two experimental days. The samples were transferred and stored at -20 °C for later analyses. Total urine was collected into plastic buckets containing 10 ml HCl (36%) twice each day during the last two days, proportionally

sampled, and then stored at -20 °C. For determining duodenal supply of bacterial amino acids (AA), ruminal digesta and fluid were sampled for isolation of ruminal bacteria after the end of sample collection during each period.

Lab Chemical Analysis Procedures

The six-hourly digesta (duodenal, ileal) and fecal samples collected during each sampling day from individual animals were mixed and proportionally pooled before freeze-drying. Sample weight before and after drying and the pooled sample volume were measured and recorded. Cr and Co concentrations in freeze-dried samples of duodenal, ileal and fecal contents were determined in duplicate by atomic absorption spectrometry (Techtron Private Ltd., Melbourne, Australia). Standards were prepared using a Cr and Co atomic absorption standard solution (Sigma Chemical Co., St. Louis, MO).

For determination of urinary N content, 5 ml of each urine sample was transferred into a pre-weighed plastic tubing, re-weighed and urinary N was analysed by the standard Kjeldahl method (AOAC, 1990).

Rumen digesta collected through the ruminal cannula was immediately centrifuged at 500 x g at 4 °C for 10 min after collection. The supernatant was centrifuged again at 30,000 x g at 4 °C for 30 min, the supernatant was then discarded, the residue pellet (bacterial fraction) was washed two times with 0.9% NaCl and centrifuged after each washing at 30,000 x g at 4 °C for 10 min. The residue pellets were freeze-dried, pooled within treatment, and stored at -30 °C until analysis (Cecava et al., 1990).

For amino acid analyses, except for cysteine and methionine, approximately 100 mg of sample was weighed into a screw-capped test tube and mixed with 3 ml of 6 N HCL. The tubes were purged with nitrogen and then hydrolyzed in an oven at 110 °C for 24 h. The hydrolyzed samples were mixed with the internal standard, DL-β-amino-n-butyric acid, and centrifuged at 1,110 g for 15 min at 4 °C. The supernatant of the sample was analyzed according to principles outlined by Jones and Gilligan (1983) and modified by Sedgwick et al. (1991) using a Varian 5000 high performance liquid chromatography system with a reverse-phase column and a Varian Fluorichrom detector (Varian Canada Inc., Mississauga, ON, Canada). The amino acids were derivatized with an o-phthaldialdehyde reagent solution.

The mobile phase consisted of two solvents with a flow rate of 1.1 ml/min. Solvent A contained 0.1 M sodium acetate (pH 7.2), methanol, and tetrahydrofuran in a ratio of 90 to 5; solvent B was pure methanol. Peaks were recorded and integrated using the Ezchrom™ Chromatography Data System (version 2.12; Shimadzu Scientific Instruments Inc., Columbia, MD, U.S.A.).

Methionine and cysteine were determined as methionine sulfone and cysteic acid after oxidation with 98% performic acid overnight according to AOAC (1990). The oxidized samples were dried according to procedures described by Dugan et al. (1992), then hydrolyzed and analyzed in the same manner as the other amino acids.

For diaminopimelic acid (DAPA) analyses, approximately 0.15 g of sample was weighed into a screw-capped test tube and mixed with 3 ml of 6 N HCL. The tubes were purged with nitrogen and then hydrolyzed in an oven at 110 °C for 24 h. The hydrolyzed samples were mixed with the internal standard, DL-α-amino-caprylic acid, and centrifuged at 1,500 × g for 15 min at 4 °C. The supernatant of the sample was analyzed according to principles outlined by Dugan et al. (1992) using a Varian 5000 high performance liquid chromatography system with a reverse-phase column and a Varian Fluorichrom detector (Varian Canada Inc., Mississauga, ON). The DAPA was derivatized with an o-phthaldialdehyde reagent solution. The mobile phase consisted of a binary gradient changing from a polar to a non-polar solvent with a flow rate of 1.1 ml/min. The polar solvent consisted of a water-methanol mixture (60:40, v/v) containing 0.1 M sodium acetate and 7.5mM hexadecyltrimethylammonium bromide (HTMA). The non-polar solvent consisted of a methanol-water mixture (95:5, v/v) containing 7.5 mM HTMA. Peaks were recorded and integrated using the Ezchrom™ Chromatography Data System (version 2.12; Shimadzu Scientific Instruments Inc., Columbia, MD).

The apparent duodenal, ileal and faecal digestibilities of dry matter (DM), organic matter (OM), crude protein (CP) and amino acids (AA) in the experimental diet were calculated by formula: Digestibility (%) = ((observed mass (g) at point A - observed mass (g) at point B)/ observed mass (g) at point A) * 100.

Statistical Analysis

Data from each experiment were statistically analyzed by the GLM procedures of SAS (1996). The model included factors of animal, infusion treatment and their interaction for all parameters from both experiments. The multiple comparison for interaction was analyzed by lsmean with Pdiff option.

C. Results

Experiment 1

Feed and water intake

Intake of DM, OM, CP and AA increased ($P < 0.05$) over 14% in response to VIP infusion (Table 3-1), compared to saline infusion. Vasoactive intestinal polypeptide infusion did not significantly influence AA and water intake (Table 3-1).

Digesta passage

Digesta passage per day at the proximal duodenum was not significantly affected by VIP treatment (Table 3-1). However, VIP infusion tended to increase the supply of DM, OM, CP and AA to duodenum. Supplies of DM, OM, CP and AA were on average 6.1, 9.2, 6.4, and 8.8% higher for VIP treated than the control animals (Table 3-1). Total bacterial AA passage estimated by DAPA and mass of bacterial AA per unit digested OM in forestomach were not changed by VIP infusion (Table 3-1). Duodenal liquid flow rate was also not influenced by infusion treatment (Table 3-1).

Over 69% of AA reaching the duodenum consisted of bacterial AA as estimated by DAPA content being 78% for VIP and 69% for saline treatment (Table 3-1). Individual AA duodenal passage was not influenced by infusion treatment (Table 3-2).

Nutrient disappearance and digestibility

Although VIP infusion intended to increase mean nutrient disappearance by 21.5%, 19.6%, 30.5% and 36.9% for DM, OM, CP and AA, respectively, these values for disappearances in the forestomach were not significantly influenced by VIP, compared to saline infusion (Table 3-1. Efficiency of microbial protein synthesis varied from 11.4 to 23.7 (g N/kg of OM disappearance in the forestomach) and was not influenced by VIP.

Disappearance of individual AA in forestomach was the same in both VIP and saline infusion treatments (Table 3-2) and there were no effects of VIP on duodenal passage of individual AA.

Experiment 2

Feed and water intake

Intake of DM, OM, CP and AA were increased ($p<0.05$) by VIP infusion (Table 3-3). The feed intake during the VIP treatment was 26.1% higher than that of the saline control group. Organic matter and CP intake were increased by 25.3% and 28.7% in response to VIP infusion. Vasoactive intestinal polypeptide infusion did not significantly influence water intake (Table 3-3).

Digesta passage

Duodenal passages of DM, OM, CP and AA were increased by 22.8%, 22.5%, 51.0% and 62.8% in VIP treatment, compared to that in saline treatment (Table 3-3). Terminal ileal passages of DM, OM, CP and AA were 20.4%, 20.4%, 70.2% and 22.9% higher in VIP treatment than that in saline treatment. However, these differences were not significant ($P > 0.5$). Duodenal liquid flow rate tended to increase ($P=0.06$) in VIP infusion. There was no difference in ileal liquid flow rate between VIP and saline infusion (Table 3-3). The increased supply of nutrients to the duodenum could not have much beneficial effect on animal performance because of simultaneous, nonsignificant, increase of AA passage to the large intestine (Tables 3-3). Individual AA passage at the duodenum (Table 3-4) and ileum (Table 3-5) was not altered by infusion treatments.

Significant increases of N intake due to VIP infusion did not result in a higher N retention than that for saline infusion because of relatively high fecal and urinary N losses (Table 3-6).

Nutrient disappearance and digestibility

Disappearance of DM and OM in the forestomach tended to increase during VIP treatment. VIP infusion increased disappearance of DM and OM by 36.5% ($P = 0.07$) and

35.1% (P = 0.08), compared to that saline infusion, respectively (Table 3-6). There was no difference in forestomach CP and AA disappearance between infusion treatments. Although VIP infusion potentially increased nutrient disappearance in the small intestine by 38.2%, 42.6%, 28.4% and 55.8% on DM, OM, CP and AA, respectively, compared to that of saline, these trends were not significantly different (Table 3-6). VIP infusion did not influence individual AA disappearances in the forestomach or passage into the duodenum (Table 3-3) or disappearance from the small intestine (Table 3-5)

Percentage disappearance of DM, OM, CP and total AA in the forestomach and the small intestine were not changed by VIP treatment (Table 3-7). The measured apparent total tract digestibility (total collection) and Cr-estimated total digestibility of nutrients were the similar (Table 3-7).

D. Discussion

The narrowness of the ROO has been considered to limit digesta flow out of the reticulo-rumen because digesta flow could be doubled by artificially keeping the orifice open (Bueno, 1972; Ruckebush, 1988). The ROO opening by the inhibitory effects of VIP gives a potential chance to increase outflow rate of reticuloruminal digesta (Reid et al., 1988a,b; Okine et al., 1995), so that VIP may play a role in up-regulation of feed intake. The present results support this hypothesis that an effect of VIP in causing the relaxation of the ROO would facilitate digesta outflow of the reticulorumen with an associated increase of feed intake. The upward regulation may not only mainly relate to lowered muscle tone of the rumen by VIP, but also a lower pressure of the omasum that is relaxed by VIP (Chapter 2) results in advantage for the reticulo-rumen digesta flow into the omasum by pressure differential and potential difference. Another possibility is that VIP may reduce the activity of series tension receptors in the ruminal wall to reduce reflex stimulation on the satiety centre in the hypothalamus and, thereby allow animal to consume more food within a certain time period.

The longer administration and higher dosage of VIP infusion in Exp.2 increased percent DM intake by 26% over that of saline infusion, compared to the increase of DM intake by only 14% in Exp.1. This, however, did not result in a significant change of

duodenal digesta flow in Exp.2, which was unexpected because the higher infusion frequency and dosage of VIP should have produced a stronger inhibition of the ROO electric activity (Reid et al., 1988a), which should have facilitated digesta outflow of ruminoreticulum. The non-significant alteration of duodenal OM, CP, AA and bacterial AA flow by VIP infusion indicates that the short-term inhibition of VIP on ruminal motility (Chapter 2) was apparently too small to affect daily synthesis of microbial protein. Although VIP increased intake, significant individual animal variations ($P<0.05$) in the response to VIP treatment may have precluded the ability to detect changes of digesta and nutrient flows in response to treatment. In addition, there is the possibility that sampling from the T-type cannula may not have always given a representative samples of whole digesta. This may also have contributed to variability (Harmon and Richards, 1997). The concentration of amino acid N in duodenal contents, ranging from 64 to 80 % of total N for the alfalfa pellet diet in the present study is quite similar to 65-76% of NAN value for pelleted diets reported by Kennedy et al. (1986), but is higher than value of about 35% of total N calculated from the results of Beever and Thomson (1981).

Relatively higher water intake in response to VIP treatment under both experiments was probably related to the increased DM intake, rather than a specific effect of VIP. The contrary tendency of duodenal liquid flow rate resulted from effect of VIP in Exp.1 (Tables 3-1) and Exp.2 (Table 3-3), compared to the saline treatment, may suggest further that study is needed for clarification of any role of VIP in modifying duodenal liquid flow. Hyun et al. (1995) found that VIP injection stimulated the jejunal secretion of water in sheep, in vitro, while Nassar et al. (1995) found that i.v. injection of VIP inhibited, in vitro, water absorption in jejunum by almost 10 % in rats. These previous studies actually suggested that there is higher ileal liquid flow rate under VIP treatment. However, the present data of ileal, in vivo, liquid flow rate in Exp.2 did not give strong evidence to support previous, in vitro, results (Hyun et al., 1995; Nassar et al., 1995). The difference may be caused by inherent differences of structure and function of the small intestine between species. Moreover, the isolated tract or anaesthetized animal may not represent what happens in the intact and conscious animals under integrative living conditions.

Two previous studies showed that VIP significantly inhibited the absorption of [^{3}H]-

leucine by 59.1% (Chen et al., 1987) and alanine by 60% (Nassar et al., 1995) in rats. The present data seems not to support these previous results because there was no difference in the disappearance of individual AA in the small intestine under infusion treatments in Exp.2. The difference may be explained by species of animal and/or the methodology used. In addition, disappearance is not synonymous with absorption because the former can not exclude chemical content from endogenous excretion in the gastrointestinal tract.

The percentage of disappearances of DM (>52%), OM (>57%) and AA (>35%) in the forestomach in Exp.1 was higher than that in Exp.2 (Table 3-7). The reason for a relatively lower digestion in the forestomach in Exp.2 is not clear. Higher dietary CP concentration in Exp.2 may be a factor contributing to the difference. However, other possible factors causing differences may include total intake, different fistula status and longer duration and higher dosage of VIP infusion. It was also noted that the relatively lower disappearance of CP and AA in the forestomach was compensated for in the small intestine in the VIP treatment (Table 3-7). A shift in site of digestion of forage OM from the rumen to the small intestine was demonstrated by Beever et al. (1985) when DM intake increased from 1.7 to 2.4% of BW in Holstein steers consuming white clover. Increased DM intake in the present studies by the effect of VIP may have caused a similar shift. Based on the present data, we speculate that lysine and cysteine are poorly digested in the small intestine of ruminants, compared to other AAs. However, histidine, tyrosine, asparagine and methionine appeared to have excellent digestion in sheep fed alfalfa pellets.

Increased dosage and infusion rate of VIP in Exp.2 did improve feed intake but had no further effect on duodenal digesta passage rate relative to Exp.1. The explanation of a lack of effect of dose rate on passage rate may be due to limited quantity of tissue receptors and/or short half-life of VIP (Reid et al., 1988a,b). In addition, availability of the reticuloruminal digesta particulate size that is physically ready to pass through ROO may have been limited and variability among animals may have prevented our ability to detect a significant treatment effects on passage. However, when duodenal DM or OM passage was regressed on intake, there was a significant ($P = 0.01$) positive regression ($Y=0.746X$ (g DM/d) - 196.61, $R^2 = 0.67$; or $Y=0.779X$ (g OM/d) - 189.57, $R^2 = 0.68$).

The Cr content of the timothy hay labelled in the present experiments (24.6 mg Cr

g^{-1} of fiber) was similar to the lowest Cr content used by Miaron and Christopherson (1992), so little effect of the marker on density and passage would be expected. The estimated total tract digestion using the external marker gave values comparable to the apparent digestion (Table 3-7) measured by total collection. However, there was a large variation in CP digestion among individual animals. Although average fecal recovery of Cr was 92% or higher, a wide range of Cr-recovery (84-105%) at the duodenum and ileum indicated that current estimated digestion by a single marker system needs to be refined to reduce this variation (by longer collection period and more sampling times). To eliminate the variance by differences in intake, adding marker to feed may be better than dosing through the rumen cannula. A longer dosing period may also yield a more stable result. A multiple marker system to more accurately estimate nutrient flow and digestion has been suggested and used in many studies, but improvement in the unbiased estimations is still being debated (Titgemeyer, 1997). In addition, the retention time of solid and fluid phases is not constant for a given feed but highly variable depending on the physiological state of the experimental animal (Weston, 1988).

In conclusion, VIP infusion stimulated an increase of DM, OM, CP, and AA intakes when sheep had access to feed over 8 h per day. Vasoactive intestinal polypeptide also tended to enhance the supply of OM and AA to duodenum for further digestion and absorption. However, VIP apparently did not induce a change of liquid fluid passage.

Table 3-1. Intake, digesta flow and nutrient disappearance in response to VIP treatment in Exp.1.

Item	VIP	Saline	Pooled SE	P
Intake (g/d)				
DM	1201.3	1051.3	42.2	0.050
OM	1079.6	945.7	32.4	0.027
CP	176.9	155.0	5.3	0.027
AA	155.5	136.1	6.7	0.028
Water (L/d)	3.9	3.7	0.7	0.726
Duodenal passage (g/d)				
DM	524.6	494.4	31.4	0.522
OM	439.1	401.8	27.9	0.514
CP	111.6	104.9	11.2	0.686
AA	88.9	86.5	9.8	0.833
Microbial AA	69.4	59.9	5.5	0.962
Microbial AA (g/kg DOM)*	111.9	112.8	20.6	0.977
Duodenal liquid flow (L/d)	14.8	16.0	0.9	0.351
Disappearance in forestomach				
DM (g/d)	660.4	543.6	56.7	0.196
OM (g/d)	650.5	544.0	51.5	0.194
CP (g/d)	65.4	50.1	15.5	0.512
AA (g/d)	64.6	47.2	13.8	0.316
Efficiency of microbial protein synthesis (g N/kg OM digested in forestomach)	17.4	20.4	2.5	0.434

Notes: Each mean value is average of four observations.

* DOM --- OM digested in forestomach.

Table 3-2. AA disappearance (g/d) in the forestomach and duodenal passage (g/d) in response to VIP treatment in Exp.1.

Item	Duodenal passage				Disappearance in stomach			
	VIP	Saline	Se*	P	VIP	Saline	SE	P
ASP	10.5	9.7	1.11	0.702	10.5	7.4	1.07	0.244
GLU	11.6	10.7	1.25	0.862	8.5	5.9	1.77	0.330
SER	3.9	3.5	0.51	0.950	3.1	2.2	0.70	0.434
HIS	1.5	1.4	0.35	0.762	2.0	1.5	0.44	0.427
GLY	6.0	5.5	0.58	0.780	4.4	3.0	0.82	0.268
THR	4.8	4.4	0.52	0.783	3.3	2.2	0.73	0.306
ARG	4.1	3.8	0.38	0.564	3.6	3.0	0.60	0.508
ALA	6.8	6.3	0.74	0.745	4.6	2.9	1.02	0.298
TYR	3.5	3.1	0.36	0.700	3.0	2.0	0.53	0.253
VAL	6.7	6.2	0.81	0.762	5.0	3.2	1.11	0.307
PHE	5.3	4.9	0.55	0.883	4.4	3.1	0.79	0.305
ISO	6.2	5.7	0.82	0.999	4.2	2.9	1.01	0.434
LEU	8.5	7.9	1.00	0.770	6.5	4.3	1.39	0.311
LYS	6.6	6.0	0.79	0.755	3.5	2.0	1.04	0.347
MET	1.3	1.2	0.14	0.864	1.3	1.0	0.17	0.265
CYS	1.6	1.4	0.10	0.630	0.9	0.6	0.21	0.245

Notes: each mean value is average of four observations.

* Se -- pooled mean standard error.

Table 3-3. Effect of VIP infusion on intake and digesta passage in Exp.2.

Item	VIP	Saline	Pooled SE	P value
Intake (g/d)				
DM	1149.4	911.8	65.0	0.019
OM	918.3	732.9	53.3	0.028
CP	239.2	185.9	15.1	0.019
AA	163.1	129.4	8.2	0.019
Water (L/d)	3.4	2.9	0.7	0.438
Duodenum passage (g/d)				
DM	630.8	513.6	89.1	0.388
OM	499.6	407.9	76.3	0.428
CP	166.2	110.1	26.8	0.189
AA	120.5	74.0	21.2	0.171
Liquid flow (L/d)	14.3	11.6	1.0	0.064
Ileum passage (g/d)				
DM	538.4	447.3	61.5	0.326
OM	421.0	350.7	47.9	0.329
CP	101.1	59.4	17.4	0.141
AA	56.8	33.9	9.8	0.137
Liquid flow (L/d)	4.9	4.2	0.4	0.225

Notes: each mean value is average of four observations.

Table 3-4. AA disappearance (g/d) in the stomach and duodenal passage (g/d) in response to VIP treatment in Exp.2.

Item	Duodenal passage				Disappearance in stomach			
	VIP	Saline	Se	P	VIP	Saline	SE	P
ASP	12.6	8.2	2.17	0.202	7.5	7.4	1.50	0.962
GLU	15.2	9.5	2.67	0.186	3.5	5.0	1.91	0.607
SER	4.9	3.1	0.84	0.195	2.6	2.6	0.56	0.934
HIS	2.1	1.2	0.43	0.194	1.5	1.6	0.32	0.847
GLY	9.0	5.5	1.56	0.163	2.8	3.7	1.10	0.594
THR	5.2	3.2	0.87	0.167	2.1	2.4	0.61	0.721
ARG	5.9	3.5	1.08	0.164	2.5	3.1	0.77	0.632
ALA	9.2	5.7	1.62	0.175	3.1	3.9	1.18	0.658
TYR	4.9	2.9	0.80	0.133	3.7	3.7	0.56	0.925
VAL	8.8	5.5	1.52	0.173	3.2	3.9	1.08	0.677
PHE	8.0	4.5	1.49	0.157	2.1	3.2	1.11	0.481
ISO	8.3	4.8	1.50	0.152	2.0	3.2	1.09	0.474
LEU	12.8	7.4	2.44	0.164	4.1	6.0	1.82	0.502
LYS	9.1	5.6	1.67	0.191	1.2	2.4	1.29	0.542
MET	2.5	1.7	0.30	0.123	1.8	1.6	0.18	0.509
CYS	2.1	1.6	0.31	0.263	0.3	0.3	0.21	0.989

Notes: each mean value is average of four observations.

Table 3-5. AA disappearance (g/d) in the small intestine and ileal passage (g/d) in response to VIP treatment in Exp.2.

Item	Ileal passage				Disappearance in small intestine			
	VIP	Saline	Se	P	VIP	Saline	SE	P
ASP	6.3	4.2	0.91	0.132	6.3	4.4	1.17	0.299
GLU	7.1	4.7	1.02	0.119	8.0	5.4	1.59	0.291
SER	2.7	1.7	0.39	0.127	2.2	1.6	0.42	0.371
HIS	1.0	0.5	0.17	0.071	1.1	0.7	0.26	0.329
GLY	5.0	3.2	0.79	0.156	3.8	2.7	0.73	0.337
THR	3.1	2.1	0.40	0.116	2.0	1.3	0.41	0.236
ARG	2.2	1.4	0.31	0.099	3.8	2.3	0.79	0.213
ALA	4.2	2.8	0.62	0.154	4.9	2.9	0.94	0.186
TYR	2.2	1.5	0.35	0.162	2.5	1.6	0.41	0.152
VAL	4.0	2.7	0.55	0.122	4.7	2.9	0.88	0.204
PHE	3.0	1.9	0.42	0.113	4.9	2.8	1.04	0.197
ISO	3.0	2.0	0.43	0.140	5.2	2.9	1.00	0.163
LEU	4.8	3.0	0.70	0.116	8.0	4.5	1.75	0.208
LYS	4.0	2.4	0.62	0.104	5.0	3.5	1.05	0.336
MET	1.0	0.8	0.14	0.313	1.5	1.0	0.17	0.100
CYS	2.3	1.7	0.29	0.148	-0.3	-0.1	0.17	0.312

Notes: each mean value is average of four observations.

Table 3-6. Nutrient disappearance and N balance in response to VIP treatment in Exp.2.

Item	VIP	Saline	Pooled SE	P value
Disappearance in forestomach (g/d)				
DM	528.6	387.4	45.5	0.071
OM	428.0	316.9	37.7	0.082
CP	73.0	75.8	18.7	0.917
AA	44.0	53.9	15.1	0.660
Disappeared in small intestine (g/d)				
DM	90.0	65.1	13.4	0.236
OM	77.7	54.5	19.9	0.419
CP	65.1	50.7	11.8	0.419
AA	63.7	40.9	12.5	0.244
N balance				
N intake (g/d)	37.9	30.1	2.1	0.019
Measured fecal N output (g/d)	12.7	8.6	2.2	0.229
Measured urinary N output (g/d)	10.6	7.8	1.7	0.262
Average N retention (g/d)	14.7	13.8	2.3	0.794

Notes: each mean value is average of four observations.

Table 3-7. Disappearance of DM, OM, CP and AA at gastrointestinal phase in response to VIP treatment in Exp.2.

Item	VIP	Saline	Pooled SE	P-Value
Total apparent digestibility (%)				
DM	59.0	56.9	4.95	0.770
OM	56.7	53.9	5.03	0.459
CP	67.9	71.7	4.10	0.570
Disappearance in forestomach (% of intake)				
DM	46.8	43.1	5.59	0.658
OM	47.5	43.9	5.93	0.679
CP	32.2	40.7	9.53	0.551
AA	29.0	41.8	10.13	0.439
Disappearance in small intestine (% of duodenal passage)				
DM	14.5	12.4	1.73	0.428
OM	15.0	12.6	3.14	0.608
CP	40.2	44.8	3.61	0.394
AA	53.5	54.1	3.54	0.915
Estimated total apparent digestion by Cr marker (%)				
DM	58.5	53.9	4.24	0.469
OM	56.2	50.8	4.26	0.393
CP	67.4	69.8	4.26	0.698

Notes: each mean value is average of four observations.

E. Literature Cited

AOAC. 1990. Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemists, Arlington, VA.

Beever, D. E., D. J. Thomson, M. J. Ulyatt, S. B. Cammel, and M. C. Spooner. 1985. The digestion of fresh perennial ryegrass (Lolium Perenne vs. Melle) and white clover (Trifolium repens cv. Blanca) by growing cattle fed in doors. Br. J. Nutr. 54: 763-775.

Beever, D. E., and D. J. Thomson. 1981. The effect of drying and processing red clover on the digestion of the energy and nitrogen moieties in the alimentary tract of sheep. Grass Forage Sci. 36: 211-219.

Bueno, L. 1972. Action du sphincter reticulo-omasal chez les bovins. Annls. Rech. Vétér. 3: 83-91.

CCAC. 1993. Guide to the Care and Use of Experimental Animals. Vol. 1. Canadian Council on Animal Care, Ottawa, ON.

Cecave, M. J., N. R. Mercjen, L. C. Gay, and L. L. Berger. 1990. Cpmposition of ruminal bacteria harvested from steers as influenced by dietary energy level, feeding frequency, and isolation techniques. J. Dairy Sci. 73: 2480-2488.

Chen, Y. F., Z. T. Feng, S. H. Wen, and G. J. Liu. 1987. Effect of vasoactive intestinal polypeptide, somatostatin, neurotensin, cholecystokinin octapeptide, and secretin on intestinal absorption of amino acid in rat. Digestive Diseases & Sciences. 32: 1125-1129.

Chikhlssa, A. R., A. Gharzouli, G. Charpin, M. Descroix-Vagne, and D. Pansu. 1992. Comparison of VIP-induced electrolyte secretion at three levels in rat small intestine. Reproduction, Nutrition, Development. 32(1):37-45.

Denac, M., C. Oertle, G. Kumin, E. Eggenberger, and E. Scharrer. 1990. Relaxation of muscle strips from the reticular groove and reticulo-omasal orifice by vasoactive intestinal peptide. J. Vet. Med., Series A 37:425-429.

Denac, M., J. Marti, and E. Scharrer. 1987. Relaxation of ruminal smooth muscle by vasoactive intestinal polypeptide. J. Vet. Med, A Anim. Physiol., Pathol. Clini. Vet. Med. 34:317-320.

Dugan, M. E. R., W. C. Sauer, and T. W. Fenton. 1992. A vortex vacuum evaporator for samples in test tubes. Can. J. Anim. Sci. 72:189.

Harmon, D. L., and C. J. Richards. 1997. Considerations for gastrointestinal calculations in ruminants. J. Anim. Sci. 75: 2248-2255.

Hernandez, D. E., C. Jennes, and S. G. Emerick, 1987. Brain vasoactive intestinal peptide: a potent stimulant of gastric acid secretion. Brain Research. 420(1):129-134.

Hyun, H. S., T. Onaga, H. Mineo, and S. Kato. 1995. Effect of vasoactive intestinal polypeptide (VIP) on the net movement of electrolytes and water and glucose absorption in the jejunal loop of sheep. Journal of Veterinary Medical Science. 57(5):865-9.

Iwabuchi, Y., and T. Masuhara T. 1995. Effects of vasoactive intestinal peptide and its homologues on the acetylcholine-mediated secretion of fluid and protein from the rat submandibular gland. General Pharmacology. 26(5):961-70.

Jones, B. N., and J. P. Gilligan. 1983. *o*-phthaldialdehyde precolumn derivitization and reverse-phase high performance liquid chromatography of polypeptide hydrolysates and physiological fluids. J. Chromatogr. 266:471.

Kelly, J. M., R. J. Christopherson, and R. J. Early. 1989. Apparent digestibility of amino acids and other nitrogenous compounds in the small intestine of wethers exposed to a cold environment. Can. J. Anim. Sci. 69: 921-929.

Kennedy, P.M., R. J. Early, R. J. Christopherson, and L. P. Milligan. 1986. Nitrogen transformations and duodenal amino acid content in sheep given four forage diets and exposed to warm and cold ambient temperatures. Can. J. Anim. Sci. 66: 951-957.

Kiela, P., R. Zabielski, P. Podgurniak, M. Midura, W. Barej, P. Gregory, and S. G. Pierzynowski. 1996. Cholecystokinin-8 and vasoactive intestinal polypeptide stimulate exocrine pancreatic secretion via duodenally mediated mechanisms in the conscious pig. Experimental Physiology. 81(3):375-84.

Lam, K. S. 1991. Vasoactive intestinal peptide in the hypothalamus and pituitary. [Review] Neuroendocrinology. 53 (Suppl 1): 45-51.

Luts, L., and F. Sandler. 1994. Peptide-containing nerve fibres in the parathyroid glands of

different species. Regulatory Peptides. 50(2): 147-158.

Miaron, J. O. O., and R. J. Christopherson. 1992. Effect prolonged thermal exposure on heat production, reticular motility, rumen-fluid and -particulate passage-rate constants, and apparent digestibility in steers. Can. J. Anim. Sci. 72(4):809-819.

Nassar, C .F., L. E. Abdallah, K. A. Barada, S. F. Atweh, and N. E. Saade. 1995. Effects of intravenous vasoactive intestinal polypeptide injection on jejunal alanine absorption and gastric acid secretion in rats. Regulatory Peptides. 55: 261-267.

Okine, E.K., J. O. O. Miaron, S. Cosgrove, and R. J. Christopherson. 1995. Vasoactive intestinal polypeptide inhibits electromyographic activity of the reticulo-omasal orifice in meal-fed sheep in warm and cold environments. Can. J. Anim. Sci. 75: 333-339.

Okine, E. K., and G. W. Mathison. 1996. Effects of vasoactive intestinal polypeptide on electromyographic activity of the reticulo-omasal orifice in sheep at two levels of intake. Can. J. Anim. Sci. 74: 1681-1688.

Reid, A.M., and D. A. Titchen. 1988. Gastric electromyographic activity in the milk-fed lamb. Journal of Physiology 396: 25-39.

Reid, A.M., A. Shulkes, and D. A. Titchen. 1988a. Effects of the vagus nerves on gastric mobility and release of vasoactive intestinal peptide in the anaethetized lamb. J. Physiol. 396:11-24.

Reid, A.M., A. Shulkes, and D. A. Titchen. 1988b. The vasoactive intestinal polypeptides on gastric motility in the lamb. J. Physiol. 396:41-45.

Rodriguez-Lopez, A. M., I. De Dios, L. J. Garcia, M. A. Lopez, and J. J. Calvo. 1995. Dose-response effects of VIP on the rabbit exocrine pancreatic secretion. Comparison with PACAP-27 actions. Revista Espanola de Fisiologia. 51(1):29-36.

Ruckebusch, Y. 1988. Motility of the gastro-intestinal tract. In: Church, D.C., ed. The Ruminant Animal. Englewood Cliffs, NJ.

Saade, N. E., L. E. Abdallah, K.A. Barada, S. F. Atweh, and C. F. Nassar. 1995. Effects of intracerebral injections of VIP on jejunal alanine absorption and gastric acid secretion in rats. Regulatory Peptides. 55: 269-276.

Said, S.I. 1984. Vasoactive intestinal polypeptide (VIP): Current status. Peptides. 5:143-150.

SAS. 1996. SAS/STAT® user's guide (Release 6.12). SAS Inst. Inc., Cary, NC.

Sedgwick, G., T. W. Fenton, and J. R. Thompson. 1991. Effect of protein precipitating agents on the recovery of plasma free amino acids. Can. J. Anim. Sci. 71:953.

Toullec, R., J. A. Chavialle, P. Guilloteau, and C. Bernard. 1992. Early-patterns of plasma gut regulatory peptide levels in calves. Effect of age, weaning and feeding. Comp. Biochem. Physiol. 102A: 203-209.

Titgemeyer, E. C. 1997. Design and interpretation of nutrient digestion studies. J. Anim. Sci. 75(8): 2235-2247.

Uden, P., P. E. Colluci, and P. J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digestion rate passage studies. J. Sci. Food Agric. 31: 625-632.

Wathuta, E. M. 1986. The distribution of vasoactive intestinal polypeptide-like, substance p-like and bombesin-like immunoreactivity in the digestive system of the sheep. Quart. J. Experim. Physiol. 71: 615-631.

Weston, R. H. 1988. Factor limiting the intake of feed by sheep. XI. The effect of pregnancy and early lactation on the digestion of a medium-quality roughage. Austr. J. Agric. Res. 39: 659-669.

Yamamoto, Y., N. Kitamura, J. Yamada, and T. Yamashita. 1994. Immunohistochemical study of the distributions of the peptide- and catecholamine-containing nerves in the omasum of the sheep. Acta Anatomica. 149(2): 104-110.

Zdon, M. J., T. E. Adrian, and I. M. Modlin, 1988. Gastric somatostatin release: evidence for direct mediation by calcitonin gene-related peptide and vasoactive intestinal peptide. Journal of Surgical Research. 44(6): 680-686.

CHAPTER FOUR

EFFECT OF VIP AND ENVIRONMENTAL TEMPERATURE ON TWENTY-FOUR HOUR FEED INTAKE, DIGESTA FLOW AND NUTRIENT DIGESTION IN SHEEP

A. Introduction

Internal and external environmental factors have a considerable effect on farm animal performance. Cold exposure stimulates the appetite and reduces retention time of digesta in the forestomach and increases ruminal motility, resulting in a depression in the extent of organic matter digestion in the forestomach (Kennedy and Milligan, 1978; Christopherson 1984; Cowan et al., 1993). However, cold exposure has little or no effect on digesta passage rate in the intestine so that there is no change in apparent efficiency of digestion post-ruminally (Christopherson, 1989).

Vasoactive intestinal polypeptide (VIP) as a neuro transmitter in the central and peripheral nervous system has been demonstrated to act on pancreatic secretion and regulate gut motility (Said 1984; Reid et al., 1988a,b; Luts and Sundler, 1994). Studies have shown that VIP immuno-reactivity is localized in the neural elements throughout the ovine gastrointestinal tract, as well as in the submaxillary, parotid and the sublingual salivary glands close to small blood vessels and the acini (Wathuta 1986; Luts and Sundler, 1994). In addition, VIP immuno-reactivity is found in adrenal glands (Cheung, 1988) and in a population of nerve fibres in the parenchyma of the parathyroid glands (Luts and Sundler, 1994). VIP-reactive fibers are also found in the pancreas, the gall bladder and the common bile and pancreatic duct (Wathuta, 1986). In the forestomachs, abomasum, and small and large intestines, VIP reactive fibers are present in the mucosa, submucosa, smooth muscle layers and the neural plexuses, and are particularly dense in the region of the reticulo-omasal orifice (ROO) and in the inner muscle layer of omasal wall (Wathuta, 1986; Yamamoto et al., 1994).

Denac et al. (1987) demonstrated that VIP caused a concentration-dependent reduction of acetylcholine-induced isotonic contraction of muscle strips and was more

effective in the muscle strips from calves than those from adult cattle. However, Toullec et al., (1992) did not find any effect of age, weaning and feeding on plasma VIP concentration. The relaxing effect of VIP seems to be mediated by VIP receptors of smooth muscle cells (Denac et al., 1990). Moreover, observations have shown that VIP is involved in the relaxation process of the ROO in fasted and milk-fed lambs (Reid et al., 1988a,b). In cold acclimatised sheep, irrespective of feeding levels, infusion of VIP through a gastric-artery catheter was associated with a marked reduction in reticular and ROO electromyographic (EMG) activities and a longer quiescence of ROO EMG activity (Okine et al., 1995). Okine et al. (1995) assumed that the suppression of EMG activity in their study was a reflection of smooth muscle relaxation. Relaxation of the ROO mediated by neurological and endocrinological systems and other determinants of passage from the rumino-reticulum might, therefore, be involved in the regulation of digesta passage rate (Okine et al., 1995; Reid et al., 1988b).

Intravenous infusion of VIP stimulated exocrine pancreatic secretion and increased the plasma levels of secretin in rat (Alonso et al., 1994). Hyun et al., (1995) found that VIP stimulated the jejunal secretion of water, sodium, potassium and bicarbonate in sheep. VIP is involved in regulation of epithelial transport in the guinea pig colon (Reddix et al., 1994). The suppressed chloride absorption in the ileum (Chikhlssa et al., 1992) and water absorption (Nassar et al., 1995) were also reported by VIP treatment. In addition, VIP significantly inhibited the absorption of [^{3}H]-leucine (Chen et al., 1987) and alanine (Nassar et al., 1995) in rat.

Our previous studies have shown that VIP stimulates feed intake when administered intermittently during 3 h of an 8 h daily feeding schedule. There was a tendency for an effect of VIP on digesta flow, however forestomach and small intestine digestion of nutrients were not significantly affected (Chapter 3). If VIP produces relaxation in gastric-intestinal muscle, especially in the ROO and regulates secretion and absorption by the gastrointestinal epithelium, it was proposed that exogenous administration of VIP might produce an effect on digesta passage and nutrient digestibility. We hypothesized that infusion of VIP through the gastric artery might regulate feed intake and digesta flow in sheep with access to feed for 24 h per day and that the response may be modified by environmental temperature.

The objective of this study was to investigate the effect of exogenous VIP infused intermittently over 3 h per day through a gastric-artery catheter on 24 h feed intake, duodenal particulate passage rate and liquid flow rate, water consumption, and amino acid (AA) passage in sheep exposed to thermoneutral and cold environmental temperatures.

B. Experimental Procedures

Animals and preparation

These experiments were performed to examine the effects of VIP and environmental temperatures on feed intake, digesta flow and digestibility. Three experiments 1, 2, and 3 involved sixteen wethers that ranged from 40-50 kg in body weight. The sheep were housed in individual pens and fed *ad libitum* with alfalfa pellets for 24 h (estimated ME 9.4 MJ kg^{-1}, CP 13.4% of DM) during all the experiments. All sheep had free access to water and salt licks. Before each of the treatment periods in all experiments, animals were shorn to 1 cm depth of fleece.

In experiment 1 (Exp.1), four sheep were surgically cannulated with ruminal and "T" type duodenal cannulas at proximal duodenum two weeks before the start of the experiment (Kennedy et al., 1986). A left gastric artery polypropylene catheter was inserted as described by Okine and Mathison (1996) at the same time as rumen cannulation. Animals were cared for according to the guidelines of Faculty Animal Policy and Welfare Committee of University of Alberta. After surgery, sheep were given an analgesic Torbugesic (1 ml per animal) for 2 d and antibiotic therapy with long acting Liquamycin LA (1 ml 10^{-1} kg of BW) for 4 days.

During the treatments of a single cross-over design, VIP (1 nmol ml^{-1}) (Product No. V3628, Sigma Chemical Co., St. Louis, Ottawa, ON) or physiological saline solution was infused (1 ml min^{-1}) through a gastric-artery catheter for 10 min of each of 3 hours on each of 4 consecutive days commencing at 9:00am, 2 min before feeding. Animals were housed in an environmental temperature of 1 ± 2 °C.

Net feed intake was calculated by difference of the amount of feed offered and residues weighed back on a daily base. Daily water consumption was measured by weighing supplied fresh tap water and discarded water residues once daily. A modification of the

procedure described by Miaron and Christopherson (1992) was used for Cr-mordanted timothy hay (Chapter 3). The markers, 18 g Cr-mordanted fibre and 3 g Co-EDTA, were administered three times daily through the rumen cannula starting one day before VIP treatment and continuing during four treatment days. Duodenal digesta samples (50-100 ml) were collected at four times per day on the fourth and fifth dosing day. The duodenal samples were collected in plastic containers with lids and transferred to a storage room at -20 °C. All duodenal samples (50 ml each sample) from the same sheep in each day were pooled and mixed thoroughly for proportional sub-sampling. Samples were dried with a freeze-dryer and stored at -30 °C until the assays were performed. Ruminal liquid for separation and measurement of the microbial fraction was collected after the last duodenal sample collection.

In experiment 2 (Exp.2) of two factorial complete cross -over design, four sheep were surgically prepared and given VIP and control treatments as described for Exp.1. However, the four sheep were consecutively housed at two environmental temperatures (21 ± 2 °C and 2 ± 2 °C) in periods 1 and 2, respectively.

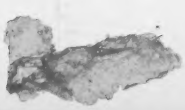
Intake of feed and water, preparation and dosing of markers, and digesta collection were the same as described for experiment 1.

In experiment 3 (Exp.3), eight sheep were surgically prepared with a gastric-artery catheter as described for experiment 1 two weeks before the start of experimental procedures. Vasoactive intestinal polypeptide (1 nmol ml^{-1}) (Product No. V3628, Sigma Chemical Co., St. Louis, Ottawa, ON) or saline was infused (1 ml min^{-1}) via the gastric artery for 10 min of each of three hours for 3 consecutive days commencing at 9:00 am, 2 min before feeding, during each of treatment periods. Animals were housed in a thermoneutral environment (21 ± 2 °C).

Feed and water intakes were calculated as described for experiment 1. The records included periods - prior to, during and after VIP infusion treatment.

Chemical analysis

Samples of diets, and duodenal digesta were ground by a Wiley mill through 0.8-mm mesh before analyses. Analyses for dry matter (DM) and organic matter (OM) were carried



out according to AOAC (1984). Crude protein was derived from total N determined with a Leco FP-428 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI).

For Co measurement, approximately 500 mg of dried duodenal sample was ashed overnight at 500 °C. Ash was dissolved in 5 ml 4 N HCl. After diluting with 15 ml deionized water, the solution was centrifuged at 180 x g for 15 min. Co concentrations in samples and standard solutions (Sigma Chemical Co., St Louis, MO 63178, USA) were analyzed by Atomic Absorption Spectrophotometer at wavelength 240.7 (4000 Atomic Absorption Spectrophotometer, Perkin-Elmer Co., Norwalk, CT 06856, USA) at a wavelength 240.7 nm.

For Cr analysis, approximately 500 mg of dried duodenal digesta was weighed, in duplicate, into screw-capped tubes and digested with 30 ml of 4M HNO_3 for 4 h at room temperature. Then, the samples were further digested in a water bath at 75 °C for 24 h. After the mixed solution was filtered, the clear sample solutions and Cr standard solutions (Sigma Chemical Co., St Louis, MO 63178, USA) were analyzed for Cr by atomic absorption at wavelength 357.9 (4000 Atomic Absorption Spectrophotometer, Perkin-Elmer Co., Norwalk, CT 06856, USA).

For amino acid analyses, except for cysteine and methionine, approximately 0.1 g of sample was weighed into a screw-capped test tube and mixed with 3 ml of 6 N HCL. The tubes were purged with nitrogen and then hydrolyzed in an oven at 110 °C for 24 h. The hydrolyzed samples were added with the internal standard, DL-amino-n-butyric acid, and centrifuged at 500 x g for 15 min at 4°C. The supernatant of the sample was analyzed according to principles outlined by Sedgwick et al. (1991) using a Varian 5000 high performance liquid chromatography system with a reverse-phase column and a Varian Fluorichrom detector (Varian Canada Inc., Mississauga, ON). The amino acids were derivatized with an o-phthaldialdehyde reagent solution. The mobile phase consisted of two solvents with a flow rate of 1.1 ml min^{-1}. Solvent A contained 0.1 M sodium acetate (pH 7.2), methanol, and tetrahydrofuran in a ratio of 90 to 5; solvent B was pure methanol. Peaks were recorded and integrated using the Ezchrom™ Chromatography Data System (version 2.12; Shimadzu Scientific Instruments Inc., Columbia, MD). Methionine and cysteine were determined as methionine sulfone and cysteic acid after oxidation with 98% performic acid overnight according to AOAC (1984). The oxidized samples were dried according to

procedures described by Dugan et al. (1992), then hydrolyzed and analyzed in the same procedure as the other amino acids.

The bacterial fraction of rumen liquid was extracted and freeze-dried (Chapter 3). Sample processing for analysis of diaminopimelic acid (DAPA) used the same procedure as common AA analysis (as described above), except for an increase in the sample size to 200 mg and use of an alternative internal standard, DL-α-amino caprylic acid. DAPA was analyzed according to principles outlined by Jones and Gilligan (1983), using a Varan 5000 HPLC system with a pre-injection guard column following procedures previously described by Dugan et al. (1989).

Statistical analysis

Experiments 1 and 3 were analysed as two treatments (VIP and saline) applied in a single factorial crossover design. Experiment 2 was a two factorial (environmental temperatures and infusion treatments) crossover design (temperature and period were confounded due to technical and scheduling constraints). The model included animals, temperatures and infusion treatments. All data are statistically analyzed by GLM procedure of SAS (1990). Mean multiple comparison for interaction from Exp.2 was completed by use of pdiff option of SAS (1990).

C. Results

Experiment 1

Infusion of VIP at 1 nmol per min did not influence feed DM, OM or AA intakes (Table 4-1) for sheep on the 24 h feeding schedule. There were no significant differences in duodenal passage rates of DM, OM, CP or AA between VIP and saline infusion treatments (Table 4-1) although there was a trend for reduced CP passage ($P < 0.10$). The ratio of bacterial N to total N content in duodenal digesta was not influenced by treatment. Microbial protein passage and efficiency of microbial synthesis were not affected by infusion treatment. There was no effect of VIP on water intake or liquid flow rate at the duodenum.

Mean duodenal flow rates of individual AA (grams per day) were not significantly different between VIP and saline infusion except for tyrosine which was reduced by VIP (P

< 0.05) (Table 4-2). There was also a trend towards reduced passage of arginine, phenylalanine and methionine (P < 0.10).

Experiment 2

Average feed DM, OM, CP and AA intakes were not significantly (P > 0.05) affected by VIP treatment (Table 4-3). VIP infusion also did not influence DM, OM, CP and AA duodenal passage. The average ratio of bacterial N to total N in duodenal digesta did not differ between VIP and saline treatments. As a result, efficiency of microbial synthesis and microbial protein passage were not altered by VIP treatment. Although VIP infusion significantly increased (P < 0.05) water intake by 20.5%, compared to that of saline infusion, liquid flow rate at the duodenum was similar between treatments (Table 4-3).

There were significant (P < 0.05) increases of DM, OM, CP and AA intakes in the cold, compared to intakes in the warm environment. There were also significant increases (p<0.05) in DM, CP, OM, AA and microbial protein passage to the duodenum in the cold, compared to these parameters in a thermoneutral environment (Table 4-4). The efficiency of microbial synthesis tended to increase (P = 0.07) in the cold environment, compared to that in the warm. There was an interaction (P < 0.05) between infusion treatment and thermal environments (Table 4-5) for duodenal passage of CP and AA. The multiple comparison by pdiff option of SAS (1990) showed that VIP infusion in the warm environment sustained (P = 0.02) CP and AA duodenal passage rate, comparable to that in the cold for VIP and saline infusion treatments. The enhanced AA passage from the reticulorumen to the duodenum by VIP tended to reduce protein degradation in rumen in the warm environment.

Duodenal flows of most individual AAs were significantly higher (p<0.05) in the cold than in the warm environment, and there was a trend for increased arginine and tyrosine flow (Table 4-6). Histidine passage was not affected by temperature. In addition, there were significant interactions between infusion treatments and environmental temperatures (p<0.05) (Table 4-7). VIP infusion sustained the AA duodenal passage in the warm environment at the same level as that in the cold environment. However, the values for the saline infusion treatment were significantly lower in the warm environment than that in the cold environment (Table 4-7). There was no interaction for histidine or tyrosine duodenal

passage.

Experiment 3

In the thermoneutral environment, VIP infusion did not influence 24 h, *ad libitum*, feed intake for the 3 days during and 4 days after infusion periods (Table 4-8). The mean intake (1734-1847 g/d) of these non-ruminally and non-duodenally cannulated sheep was similar to intakes by the cannulated sheep in the thermoneutral environment in experiment 2.

D. Discussion

Effects of VIP

Infusion of VIP under both warm and cold environments did not stimulate an increase of feed intake or AA intake (Tables 4-1, 4-3 and 4-8) when sheep had access to feed for 24 h per day. Extending duration of feed available to 24 h from the 8 h used in previous experiments (Chapter 3) appeared to reduce the likelihood of observing an effect of the brief intermittent infusions of VIP on intake. There is evidence that VIP stimulates the secretion of prolactin, growth hormone and thyroid hormone (Zudenigo and Lackovic, 1989; Lam, 1991), and relaxes the ROO (Chapter 2; Okine et al., 1995, 1996) and, therefore, has the potential to stimulate feeding in a complex way including via the central release of important hormones and/or via a local effect on the reticulorumen motility. However, our previous and present results indicate that the regulation of feeding behaviour in response to VIP did not likely involve effects on a central ingestion center because current infusion rate would not reach the physiological level in general circulation observed (262 pmol/L in gastric venous plasma) by Reid et al (1988b) besides clearance in the liver and lung (Bernhard et al., 1989). On the other hand, the significant effect of VIP on 8 h feed intakes observed in previous studies suggests that its effect may be of short duration because the feeding stimulation was apparently not sustained for the longer feeding duration of 24 h. Short-term effects of VIP include a longer opening duration of the ROO (Chapter 2) with potential effects on digesta passage (Mathison et al., 1995). Okine et al. (1995) reported the forestomach relaxation by VIP disappeared in about 90 s after stopping infusion. Much shorter duration of action was

found in our recent study (Chapter 2). The short half-life of VIP likely limits its influence on gastrointestinal motility, digesta flow and intake behaviours to a relatively short term (i.e. to the period of infusion or release). During the experiments in which feed availability was limited to 8 h per day (Chapter 3), the duration of VIP administration (intermittent infusion over 3-4 h per day) covered a large proportion of available feeding time and produced a significant change in intake. In the current experiments, the sheep were apparently able to compensate by having access to feed for the additional 16 h per day. Data from more prolonged observations also indicated that VIP infusion did not have any delayed effect on average daily feed intake over periods of up to 4 days (Table 4-8). These non-significant effects of VIP on nutrient duodenal passage could be related to the short half-life (Brook et al., 1988; Bernhard et al., 1989) and short-lived action (Reid et al., 1988a,b; Chapter 2) of VIP. However, particulate DM passage into the duodenum accounted for 43-66% of DM intake and tended to increase as feed intake increased in the present experiments, which is consistent with previous findings (Hogan, 1975; Kennedy, 1985; Chapter 3).

Water consumption was not affected by VIP in Exp.1. This was similar to results presented in one of our previous reports (Chapter 3). However, VIP infusion significantly increased water consumption in Exp.2. These variable results on water intake among our studies may be due to differences in feeding levels and physiological status (Ruckebusch et al., 1991) in different experiments. There is also a possible interaction of VIP and thermal environments (Chapter 2). The potential of VIP to stimulate submandibular salivary gland secretion (Iwabuchi and Masuhara, 1995) and decreases in water absorption by VIP (Hyun et al., 1995) may have caused thirsty signals to induce more water intake to meet requirements under conditions of Exp.2. Unfortunately, the variable results on water consumption in this study could not be used to clarify whether or not VIP is engaged in the regulation of water balance. Considering the lack of effect on duodenal liquid flow rate (Tables 4-1 and 4-3), the regulatory role of VIP on water dynamics in the digestive tract appears to be limited.

Effects of temperature

Environment and period were confounded in this study in order to facilitate

scheduling and intensive sampling and monitoring required. Although we can not rule out the possibility that some of the effects attributed to temperature might have been due to period effects, the responses reported here are consistent with previous reports of temperature effects on digestion (Kennedy et al., 1985; Miaron and Christopherson, 1992).

The cold environmental exposure-stimulated increases of feed intake and AA intake, compared to intakes in the warm environment, are consistent with previous studies (Westra and Christopherson, 1976; Kennedy, 1985; Sun et al., 1994) with wethers. However, other researchers did not find an effect of cold stimulation on intake in rams (Sano et al., 1994) and in Mountain sheep (Chappel and Hudson; 1978). The differing responses may be due to differences in species, breed, sex and fleece coat status. Results from pervious experiments of this laboratory have suggested an increased rate of passage of dietary residues from the rumen during cold exposure of sheep fed restricted levels of feed (Westra and Christopherson, 1976; Kennedy, 1985). The present study with *ad libitum* -fed sheep showed that there were significant increases ($p<0.05$) of DM, CP, OM, AA and microbial protein passage in the cold, compared to these parameters in a thermoneutral environment. Although other similar results have been reported in animals on restricted intakes (Kennedy and Milligan, 1978; Kelly et al., 1989), this is the first comprehensive study of this type in animals fed *ad libitum*. The increased passage of nutrients into the small intestine is presumably related to enhanced forestomach motility (Titchen, 1968; Christensen et al., 1991; Marion and Christopherson, 1992; Chapter 2), but it may also be secondary to increased feed intake induced by the energy demand of the environment.

An enhanced digesta flow often improves synthetic efficiency of ruminal bacteria (Kennedy et al., 1976; Kennedy and Milligan, 1978; Mathison et al., 1995). Indeed, the increased ($P < 0.05$) quantitative contributions of microbial protein to the duodenal AA supply was realised by higher digesta flow induced by cold exposure in present study. The estimates of the concentration of amino acid N in duodenal contents, ranging from 69 to 90% of total N for the alfalfa pellet diet is slightly higher than the values (65-76%) reported by Kennedy et al. (1986). Kennedy and Milligan (1978) thought that the overall consequence exposing animals to cold could increase digestible organic matter intake by about 4% when food intake is not restricted, but that the food consumed would be digested at lower

efficiency than by animals in warm conditions. In agreement with the latter, ruminal percentage digestion of DM in the warm was 14.3 percentage units higher than that in the cold. In addition, compared to the warm environment, the slightly lower ratio of duodenal bacterial N to total N in the cold implies that more nutrients escaped microbial degradation in the reticulo-rumen, and therefore, were available to the host directly from dietary material. This change was not as large as that reported by Kennedy et al. (1976) although improvements in efficiency of microbial protein synthesis were consistent with the latter report. The bacterial N contributed an average of over 63% of total nitrogen in duodenal digesta with alfalfa pellets in our experiments. This is comparable to the data of Weller et al. (1966) which indicated that bacterial N contributed on the average 62 percent of the total nitrogen in rumen contents with a low-protein diet.

Miaron and Christopherson (1992) used the fixed-intake feeding strategy to avoid possible confounding effects of differences in feeding level with environment and they found a significant increase of water intake in steers in the warm environment (10 and 28 °C), compared to that in the cold environment (-10 °C). Decreasing water consumption in sheep with decreasing temperature has also been reported in other studies (Westra and Christopherson, 1976; Thompson and Thomson, 1977). The increased water intake in the cold in the present study was likely related to higher feed intake in this environment. There is a well-established positive correlation between feed intake and water intake (Hicks et al., 1988).

In a warm environment digesta has a longer retention time in the forestomach (McBride and Christopherson, 1984; Christopherson, 1989; Miaron and Christopherson, 1992), so dietary AA degradation in reticulorumen may have increased. However, the enhanced AA passage from reticulorumen to duodenum by VIP infusion under the warm environment may have reduced protein degradation in rumen in the present study. Therefore, CP and AA supply to duodenum were sustained.

The present result that the cold exposure stimulated an increase in the duodenal flow of individual AA (Table 4-6) is consistent with the results of Kennedy et al. (1986), in which the concentration of eight amino acids (threonine, valine, isoleucine, leucine, serine, proline, glycine and alanine) were significantly increased by cold exposure in sheep fed chopped

forage and pelleted diets, although sheep were fed alfalfa pellets in present study. The significant interactions between infusion treatments and environmental temperatures in Exp.2 indicated that VIP infusion sustained the AA duodenal passage in the warm environment at a level which approached that in the cold environment. The increased supply of protein and amino acids to the duodenum in the cold environment, in conjunction with the reduced OM disappearance in the rumen, indicates that the end products of digestion are enriched in amino acids relative to energy when animals are fed *ad libitum* in a cold environment. This has the implication that sheep (and likely other ruminants) could be provided with diets formulated to contain lower crude protein concentrations during periods of cold weather. Such a practice could reduce feed costs during the winter.

In conclusion, this study demonstrated that a short duration of VIP infusion did not affect feed intake when sheep had continuous access to feed for 24 h per day and also did not influence duodenal passage of DM, OM, CP, AA and bacterial protein. The exposure of animals to a cold environment stimulated feed intake and AA intake, and duodenal passage of DM, AA and microbial protein and the efficiency of rumen microbial protein synthesis. There was an interaction of VIP treatment and environmental temperature such that VIP increased duodenal AA passage in the warm, but not the cold environment.

TABLE 4-1. Effects of VIP infusion on nutrient intake and duodenal passage in Exp.1.

Item	Treatment		SE	P
	VIP	Saline		
Feed intake (g DM/d)	2066.5	2127.3	168.2	0.539
OM intake (g/d)	1885.1	1938.1	198.9	0.860
CP intake (g/d)	308.9	317.6	32.6	0.860
AA intake (g/d)	255.3	260.4	28.4	0.906
Water intake (g/d)	3817.6	4365.9	332.5	0.254
DM duodenal passage (g/d)	898.2	1099.3	81.5	0.157
OM duodenal passage (g/d)	768.7	945.1	72.7	0.161
CP duodenal passage (g/d)	157.8	228.3	19.2	0.098
Ratio of $N_B : N_T$ [z]	77.6	67.8	6.8	0.424
AA duodenal passage (g/d)	160.2	185.2	9.8	0.145
Microbial protein duodenal passage (g/d)	136.3	149.6	7.2	0.318
Efficiency of microbial synthesis *	19.3	24.5	2.3	0.173
Liquid flow rate (L/d)	23.9	25.3	1.6	0.546
DM forestomach disappearance (g/d)	1170.5	1028.0	159.1	0.561
OM forestomach disappearance (g/d)	1116.4	993.0	45.2	0.580
CP forestomach disappearance (g/d)	131.6	89.3	17.1	0.041
AA forestomach disappearance (g/d)	92.9	75.2	18.52	0.534

Notes: each mean value in the table is the average of four observations.

* unit is gram N per kg OM digested in the forestomach.

[z] N_B - Nitrogen from bacteria in duodenal digesta; N_T - total nitrogen in digesta.

Table 4-2. Mean flow (g/d) of AA into duodenum of sheep infused VIP or saline in Exp.1.

Amino Acid	Treatment		SE	P
	VIP	Saline		
ASP	19.0	21.6	1.12	0.176
GLU	21.3	24.0	1.18	0.172
SER	7.0	8.5	0.55	0.131
HIS	3.0	3.7	0.25	0.117
GLY	10.8	12.7	0.65	0.107
THR	8.9	10.0	0.58	0.257
ARG	8.0	9.5	0.39	0.056*
ALA	12.5	13.9	0.79	0.266
TYR	5.5^{b}	6.7^{a}	0.30	0.046
VAL	11.8	13.7	0.73	0.147
PHE	9.7	11.3	0.51	0.097^{Z}
ISO	11.1	13.2	1.26	0.313
LEU	15.2	17.6	0.91	0.139
LYS	11.6	13.0	0.86	0.322
CYS	2.0	2.4	0.16	0.132
MET	2.8	3.4	0.18	0.075^{Z}

Notes: each mean value in the table is the average of four observations.

a, b means different at $\alpha < 0.05$ in the same row with different superscripts.

^{Z} means differ at $\alpha < 0.10$.

TABLE 4-3. Intake and duodenal flow of nutrients in sheep infused with VIP in Exp.2.

Item	Treatment		SE	P
	VIP	Saline		
Feed intake (g DM/d)	2145.3	2052.1	57.7	0.336
OM intake (g/d)	1954.6	1869.6	52.5	0.336
CP intake (g/d)	320.3	306.4	8.6	0.336
AA intake (g/d)	255.8	244.4	6.9	0.586
Water intake (g/d)	5945.9[a]	4935.9[b]	236.1	0.004
DM passage (g/d)	1425.0	1348.5	48.6	0.162
OM passage (g/d)	1264.2	1189.0	38.7	0.263
CP passage (g/d)	304.2	262.8	17.6	0.196
Ratio of $N_B : N_T$ [Z]	63.2	61.2	5.3	0.793
AA passage (g/d)	222.2	197.0	18.9	0.217
Microbial protein passage (g/d)	174.9	163.0	11.1	0.304
Efficiency of microbial synthesis [Y]	42.1	40.7	5.9	0.879
Liquid flow rate (L/d)	30.5	31.1	3.6	0.917

Notes: each mean value in the table is the average of eight observations.

a, b means different at $\alpha < 0.01$ in the same row with different superscripts.

Z N_B - Nitrogen from bacteria in duodenal digesta; N_T - total nitrogen in digesta.

Y unit is gram N per kg OM digested in the forestomach.

Table 4-4. Mean value of feed intake and DM passage and N passage into the duodenum in sheep exposed to different environmental temperatures in Exp.2.

Item	Temperature		SE	P
	Warm	Cold		
Feed intake (g DM/d)	1760.6[b]	2436.8[a]	57.7	0.004
Water intake (g/d)	4759.4[b]	6122.5[a]	236.1	0.001
CP intake (g/d)	262.9[b]	363.9[a]	8.6	0.004
OM intake (g/d)	1604.0[b]	2220.2[a]	52.5	0.004
Duodenal liquid flow (L/d)	20.3[b]	41.3[a]	3.6	0.002
DM duodenal passage (g/d)	1017.1[b]	1756.3[a]	48.6	0.001
OM duodenal passage (g/d)	835.5[b]	1617.7[a]	38.7	0.001
CP duodenal passage (g/d)	220.4[b]	346.5[a]	17.6	0.015
AA intake (g/d)	219.1[b]	280.6[a]	6.9	0.008
Ratio of $N_B : N_T$ (%)[Z]	65.5	59.0	5.3	0.406
AA duodenal passage (g/d)	174.4[b]	244.8[a]	18.9	0.023
Microbial protein passage (g/d)	133.9[b]	204.0[a]	6.8	0.001
Efficiency of microbial synthesis[Y]	29.8	53.0	5.9	0.069

Notes: each mean value in the table is the average of four observations.

a, b----mean significant different in the same row with the different letter.

Z N_B - Nitrogen from bacteria in duodenal digesta; N_T - total nitrogen in digesta.

Y unit is gram N per kg OM digested in forestomach.

TABLE 4-5. Interaction of infusion and environmental temperatures on intake, DM passage, liquid flow and N passage.

Item	Cold*VIP	Cold*Sal	Warm*VIP	Warm*Sa l	SE
Feed intake (g DM/d)	2498.8 [a]	2374.8 [a]	1791.9 [b]	1729.2 [b]	166.7
OM intake (g/d)	2276.6 [a]	2163.8 [a]	1632.6 [b]	1575.5 [b]	74.3
CP intake (g/d)	373.1 [a]	354.6 [a]	267.6 [b]	258.2 [b]	12.2
DM duodenal passage (g/d)	1760.9 [a]	1751.8 [a]	1089.1 [b]	945.1 [b]	150.6
OM duodenal passage (g/d)	1620.2 [a]	1615.2 [a]	908.2 [b]	762.8 [b]	54.7
CP duodenal passage (g/d)	352.6 [a]	340.5 [a]	255.8 [ab]	185.1 [b]	24.9
Duodenal liquid flow (L/d)	40.6 [a]	41.9 [a]	20.4 [b]	20.2 [b]	5.2
AA intake (g/d)	287.7 [a]	273.5 [a]	223.0 [b]	215.2 [b]	19.6
Ratio of $N_B : N_T$ (%) [Z]	57.8 [a]	60.3 [a]	64.7 [a]	66.2 [a]	6.5
AA duodenal passage (g/d)	245.8 [a]	243.8 [a]	198.7 [ab]	150.2 [b]	26.8
Microbial protein passage (g/d)	202.6 [a]	205.4 [a]	147.2 [b]	120.5 [b]	15.8
Efficiency of microbial synthesis [Y]	48.8	57.1	35.3	24.3	8.3

Notes: each mean value in the table is the average of four observations.

a, b means with the same superscription are not significantly different in the same row ($P > 0.05$).

Z N_B - Nitrogen from bacteria in duodenal digesta; N_T - total nitrogen in digesta.

Y unit is gram N per kg OM digested in forestomach.

Table 4-6. Mean passage (g/d) of AA into the duodenum of sheep at different temperatures in Exp.2.

AA	Treatment		SE	P
	Warm	Cold		
ASP	20.6	30.1	2.39	0.013
GLU	23.0	31.7	2.47	0.017
SER	7.6	11.2	0.88	0.015
HIS	4.2	4.9	0.83	0.509
GLY	11.6	16.8	1.23	0.017
THR	7.8	13.2	1.13	0.033
ARG	8.6	11.5	0.98	0.051
ALA	13.4	18.6	1.48	0.020
TYR	6.2	8.1	0.64	0.053
VAL	13.3	18.3	1.54	0.032
PHE	10.7	14.6	1.18	0.025
ISO	12.0	16.9	1.33	0.111
LEU	17.1	22.9	1.93	0.038
LYS	13.2	18.0	1.52	0.045
CYS	2.4	3.7	0.27	0.005
MET	2.9	4.3	0.32	0.001

Notes: each mean value in the table is the average of four observations.

Table 4-7. Interaction of VIP and environmental temperatures on duodenal passage (g/d) of AA in Exp.2.

AA	Interaction of Treatments				Pooled
	VIP*Cold	Saline*Cold	VIP*Warm	Saline*Warm	SE
ASP	30.7^{a}	29.5^{a}	23.4^{ab}	17.7^{b}	3.38
GLU	31.7^{a}	31.7^{a}	26.0^{ab}	19.9^{b}	3.50
SER	11.3^{a}	11.2^{a}	8.8^{ab}	6.5^{b}	1.24
HIS	4.8	5.0	5.6	2.7	1.18
GLY	6.8^{a}	16.8^{a}	13.1^{ab}	10.0^{b}	1.74
THR	13.0^{a}	13.3^{a}	7.5^{b}	8.1^{b}	1.60
ARG	11.3^{ab}	11.7^{a}	9.9^{ab}	7.3^{b}	1.39
ALA	18.8^{a}	18.5^{a}	15.5^{ab}	11.4^{b}	2.09
TYR	7.9	8.2	6.8	5.5	0.90
VAL	18.4^{a}	18.2^{a}	15.4^{ab}	11.2^{b}	2.18
PHE	14.6^{a}	14.6^{a}	12.4^{ab}	9.1^{b}	1.67
ISO	17.2^{a}	16.5^{a}	13.5^{ab}	10.5^{b}	1.88
LEU	23.0^{a}	22.9^{a}	19.9^{ab}	14.3^{b}	2.73
LYS	17.9^{a}	18.0^{a}	15.4^{ab}	11.1^{b}	2.16
CYS	3.9^{a}	3.6^{ab}	2.6^{bc}	2.1^{c}	0.38
MET	4.5^{a}	4.1^{ab}	3.0^{b}	2.7^{b}	0.45

Notes: each mean value in the table is the average of four observations.

a, b means different at α=0.05 in the same row with different superscripts.

TABLE 4-8. Feed intake (g DM/d) of 3 d average prior to, during and after VIP infusion in sheep in Exp.3.

Treatment	Period			Pooled SE	P
	Prior to	During	After		
VIP	1795.1	1734.0	1775.4	101.1	0.790
Saline	1760.9	1810.1	1847.4	101.1	0.790

Notes: each mean value in the table is the average of eight observations.

E. Literature Cited

Alonso, R.M., A. M. Rodriguez, L. J. Garcia, M. A. Lopez, and J. J. Calvo. 1994. Comparison between the effects of VIP and the novel peptide PACAP on the exocrine pancreatic secretion of the rat. Pancreas. 9(1):123--128.

AOAC (Association of Official Analytical Chemists). 1984. Official Methods of Analysis. 14th Ed. AOAC, Washington, DC.

Bernhard, W., E. Bothe-Sandtfort, H. Koop, W. Cassel, and P. Von Wichert. 1989. Degradation of vasoactive intestinal peptide by isolated, ventilated, and perfused rat lungs. Europ. J. Clin. Invest. 19(6): 506-13.

Brook, C. W., R. B. Sewell, A. Shulkes, and R. A. Smallwood. 1988. Hepatic metabolism of vasoactive intestinal polypeptide (VIP) in the rat. Regulatory Peptides. 20(4): 311-22.

Chappel, R. W., and R. J. Hudson. 1978. Winter bioenergetics of Rocky Mountain bighorn sheep. Can. J. Zool. 56 (11): 2388-2393.

Chen, Y. F., Z. T. Feng, S. H. Wen, and G. J. Liu. 1987. Effect of vasoactive intestinal polypeptide, somatostatin, neurotensin, cholecystokinin octapeptide, and secretin on intestinal absorption of amino acid in rat. Digestive Diseases & Sciences. 32: 1125-1129.

Cheung, C.Y. 1988. Ontogeny of adrenal VIP content and release from adrenocortical cells in the ovine fetus. Peptides. 9:107-111.

Chikhlssa, A. R., A. Gharzouli. G. Charpin. M. Descroix-Vagne, and D. Pansu. 1992. Comparison of VIP-induced electrolyte secretion at three levels in rat small intestine. Reproduction, Nutrition, Development. 32(1): 37-45.

Christensen, R. A., J. J. Kennelly, and R. J. Christopherson. 1991. Gastrointestinal motility and plasma hormone and metabolite response to acute cold stress and somatostatin-14 in sheep. Can. J. Anim. Sci. 71: 1079-1086.

Christopherson, R. J. 1984. Effects of cold environment on digesta and feed intake of ruminants. Farming for future, project:81-0022 (internal reference)

Christopherson, R. J. 1989. In Proceedings of the First International Symposium on Agricultural Technique for Cold Regions, Obihiro, pp. 81--89.

Cowan, R. T., R. J. Moss, and D. V. Kerr. 1993. Northern dairy feedbase 2001:2. Summer feeding system. Tropical Grasslands. 27(3):150-161.

Denac, M., C. Oertle, G. Kumin, E. Eggenberger, and E. Scharrer. 1990. Relaxation of muscle strips from the reticular groove and reticulo-omasal orifice by vasoactive intestinal peptide. J. Vet. Med., Series A 37:425--429.

Denac, M., J. Marti, and E. Scharrer. 1987. Relaxation of ruminal smooth muscle by vasoactive intestinal polypeptide. J. Vet. Med, A Anim. Physiol., Pathol.Clini. Vet. Med. 34:317--320.

Dugan, M. E. R., W. C. Sauer, and T. W. Fenton. 1992. A vortex vacuum evaporator for samples in test tubes. Can. J. Anim. Sci. 72:189.

Dugan, M.E.R., W. C. Sauer, and P. H. Robinson. 1989. Cellulose clean-up and high performance liquid chromatography of DL-diaminopimelic acid in hydrolysates of physiological samples. J. Chromatogr. 496:430--434.

Hicks, R. B., F. N. Owens, D. R. Gill, J. J. Martin, and C. A. Stasia. 1988. Water intake by feedlot steers. Okla. Anim. Sci. Rpt. Mp-125:208.

Hogan, J. P. 1975. Quantitative aspects of nitrogen utilization in ruminants. J. Dairy. Sci. 58: 1164-1677.

Hyun, H.S., T. Onaga, H. Mineo, and S. Kato. 1995. Effect of vasoactive intestinal polypeptide (VIP) on the movement of electrolytes and water and glucose absorption in the jejunal loop of sheep. J. Vet. Med. Sci. 67(5):865-869.

Iwabuchi, Y., and T. Masuhara. 1995. Effects of vasoactive intestinal peptide and its homologues on the acetylcholine-mediated secretion of fluid and protein from the rat submandibular gland. General Pharmacology. 26(5): 961-70.

Jones, B. N., and J. P. Gilligan. 1983. o-phthaldialdehyde precolumn derivatization and reverse-phase high-performance liquid chromatography of polypeptide hydrolysates and physiological fluids. J. Chromatogr. 266:471.

Kelly, J. M., R. J. Christopherson, and R. J. Early. 1989. Apparent digestibility of amino acids and other nitrogenous compounds in the small intestine of wethers exposed to a cold environment. Can. J. Anim. Sci. 69(4): 921-929.

Kennedy, P. M. 1985. Influence of cold exposure on digestion of organic matter, rates of

passage of digesta in the gastrointestinal tract, and feeding and rumination behaviour in sheep given four forage diets in the chopped, or ground and pelleted form. Br. J. Nutr. 53:159--173.

Kennedy, P. M., and L. P. Milligan. 1978. Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. Br. J. Nutr. 39:105--117.

Kennedy, P. M., R. J. Christopherson, and L. P. Milligan. 1976. The effect of cold exposure of sheep on digestion, rumen turnover time and efficiency of microbial synthesis. Brit. J. Nutr. 36: 231.

Kennedy, P. M., R. J. Early, R. J. Christopherson, and L. P. Milligan. 1986. Nitrogen transformations and duodenal amino acid content in sheep given four forage diets and exposed to warm and cold ambient temperatures. Can. J. Anim. Sci. 66:951--957.

Lam, K. S. 1991. Vasoactive intestinal peptide in the hypothalamus and pituitary. [Review] Neuroendocrinology. 53 (Suppl 1): 45-51.

Luts, L., and F. Sandler. 1994. Peptide-containing nerve fibres in the parathyroid glands of different species. Regulatory Peptides. 50(2): 147-158.

Mathison, G. W., E. K. Okine, A. S. Vaage, M. Kasake, and L. P. Milligan. 1995. Current understanding of contribution of the propulsive activities in the forestomach to flow of digesta. In: W. V. Engelhardt, S. Leonhard-Marek, G. Breves, and D. Giesecke (Ed.) Ruminant Physiology: Digestion, metabolism, Growth and Reproduction. pp 23-41. Ferdinand Enke Verlag Stuttgard, Germany.

McBride, G. E., and R. J. Christopherson. 1984. Effects of cold exposure on young growing lambs. Can. J. Anim. Sci. 64: 403--410.

Miaron, J. O. O., and R. J. Christopherson. 1992. Effect of prolonged thermal exposure on heat production, reticular motility, rumen-fluid and- -particulate passage-rate constants, and apparent digestibility in steer. Can. J. Anim. Sci. 72(4): 809-819.

Nassar, C. F., L. E. Abdallah, K. A. Barada, S. F. Atweh, and N. E. Saade. 1995. Effects of intravenous vasoactive intestinal peptide injection on jejunal alanine absorption and gastric acid secretion in rats. Regulatory Peptides. 55(3): 261-7.

Okine, E. K., and G. W. Mathison. 1996. Effects of vasoactive intestinal polypeptide on electromyographic activity of the reticulo-omasal orifice in sheep at two levels of

intake. Can. J. Anim. Sci. 74:1681--1688.

Okine, E. K., J. O. Ole Miaron, S. Cosgrove, and R. J. Christopherson. 1995. Vasoactive intestinal polypeptide inhibits electromyographic activity of the reticulo-omasal orifice in meal-fed sheep in warm and cold environment. Can. J. Anim. Sci. 75:333.

Reddix, R., A. Kuhawara, L. Wallace, and H. J. Cooke.1994. Vasoactive intestinal polypeptide: a transmitter in submucous neurons mediating secretion in guinea pig distal colon. Journal of Pharmacology & Experimental Therapeutics. 269(3): 1124-9.

Reid, A. M., A. Shulkes, and D. A. Titchen. 1988a. Effects of the vagus nerves on gastric mobility and release of vasoactive intestinal peptide in the anaethetized lamb. J. Physiol. 396:11--24.

Reid, A. M., A. Shulkes, and D. A. Titchen. 1988b. The vasoactive intestinal polypeptides on gastric motility in the lamb. J. Physiol. 396:41--45.

Ruckebusch, Y., L. P. Phaneuf, and R. Dunlop 1991. Physiology of Small and large Animals. B. C. Decker Inc. Hamilton, Ontario.

Said, S.I. 1984. Vasoactive intestinal polypeptide (VIP): Current status. Peptides. 5:143--150.

Sano, H, H. Takaya, N. Hasemi, and Y. Terashima. 1994. Effects of cold exposure and intravenous glucose infusion on feed intake and plasma insulin response to feeding in sheep. Animal Science and Technology. 65(1): 1-8.

SAS Institute, Inc. 1990. SAS/STAT user's guide (version 6.03). SAS Inst. Inc., Cary, NC.

Sedgwick, G., T. W. Fenton, and J. R. Thompson. 1991. Effect of protein precipitating agents on the recovery of plasma free amino acids. Can. J. Anim. Sci. 71:953--957.

Sun, Y. X., J. Lee, P. M. Harris, E. R. Sinclair, I. D. Shelton, H. T. Blair and S. N. McCutcheon. 1994. Nitrogen and sulfur metabolism and plasma thyroid hormone concentrations in fleeceweight-selected and control Romney sheep at two ambient temperatures. Austr. J. Agri. Res. 45(2): 339-354.

Thompson, G. E., and E. M. Thomson. 1977. Effect of cold exposure on mammary circulation, oxygen consumption and milk secretion in the goat. J. Physiol. 272: 187-196.

Titchen, D. A. 1968. Nervous control of motility of the forestomachs of ruminants. Pages 2705-2724 *in* handbook of physiology. Sec. 6. Alimentary canal. Vol. V. Am.

Physiol. Soc., Washington, DC.

Toullec, R., J. A. Chavialle, P. Guilloteau, and C. Bernard 1992. Early-patterns of plasma gut regulatory peptide levels in calves. Effect of age, weaning and feeding. Comp. Biochem. physiol. 102A: 203--209.

Wathuta, E. M. 1986. The distribution of vasoactive intestinal polypeptide-like, substance P-like and bombesin-like immunoreactivity in the digestive system of the sheep. Quart. J. Experim. Physiol. 71:615--631.

Weller, R. A., A. F. Pilgrim, and F. V. Gary. 1966. Digestion of feed stuffs in the rumen of the sheep and the passage of digesta through its compartments. 3. the progress of nitrogen digestion. Br. J. Nutr. 16:83--90.

Westra, R., and R. J. Christopherson. 1976. Effects of cold on digestibility, retention time of digesta, reticulum motility and thyroid hormone in sheep. Can. J. Anim. Sci. 56:699--708.

Yamamoto, Y., N. Kitamura, J. Yamada, and T. Yamashita. 1994. Immunohistochemical study of the distributions of the peptide- and catecholamine-containing nerves in the omasum of the sheep. Acta Anatomica. 149(2): 104-110.

Zudenigo, D., and Z. Lackovic. 1989. Vasoactive intestinal polypeptide: a potential neurotransmitter. Lijecnicki Vjesnik. 111(9-10): 354-9.

CHAPTER FIVE

EFFECT OF VIP AND ENVIRONMENTAL TEMPERATURES ON METABOLIC RATE, HEART RATE AND RESPIRATORY RATE IN SHEEP

A. Introduction

The metabolic rate is influenced by many factors including age, feeding level, diet and environment. During cold exposure metabolic rate is elevated in Bighorn sheep (Chappel and Hudson, 1978; Delfino and Mathison, 1991), lactating ewes (McBride and Christopherson, 1984), steers (Miaron, et al., 1995) and cows (McGuire et al., 1989). Respiratory frequencies (Young, 1975), rectal temperature (Horton, 1981; Liamas-Lamas and Combs, 1990) and skin temperatures (Young, 1975) are decreased in cold-exposed sheep. In contrast, the exposure to high environmental temperature may reduce heat production in sheep (Achmadi et al., 1994) and increase respiratory rate and rectal temperature in lambs (Llamas-Lamas and Combs, 1990; Bunting et al., 1992) and cows (McGuire et al., 1989).

Vasoactive intestinal polypeptide (VIP) is a neurotransmitter and is distributed in neuronal localizations in the central and peripheral nervous system (Molinero et al., 1985; Wakade et al., 1991; Chakder and Rattan, 1995; Ottesen and Fahrenkrug, 1995). Nerves immunoreactive to VIP are found in blood vessels, heart, lung, digestive and genitourinary tract, ovaries, and thyroid gland (Zimmerman et al., 1988; Zudenigo and Lackovic, 1989; Chevendra and Weaver, 1992). VIP delivery to active sites may be from the circulation or it may be released locally from nerve terminals (Blank et al., 1988). The physiological effects of VIP in the central nervous system includes the co-ordination among energy metabolism, blood flow and neuronal activity in the cerebral cortex, especially bipolar cells. VIP provokes the excitation, vasodilatation and, together with noradrenaline, participates in the regulation of cortical energy metabolism in the telencephalic areas of the central nervous system (McCulloch and Edvinsson, 1980; Zudenigo and Lackovic, 1989). Furthermore, in the brain, VIP plays a role in circadian rhythms and melatonin and pituitary hormone secretion (Simonneaux et al., 1990). The biologic effects of VIP in the periphery include vasodilatation, relaxation of smooth muscle and influences on exocrine gland secretion

(Zudenigo and Lackovic, 1989; Fahrenkrug, 1989). Effects on endocrine glands include secretion of prolactin, growth hormone, ACTH, oxytocin, vasopressin, ovarian and thyroid hormones (Zudenigo and Lackovic, 1989; Lam, 1991). Hence, the physological effects of VIP can be complex and widespread.

In the cardiovascular system, exogenous VIP infusion significantly increased heart rate, coronary blood flow and myocardial oxygen consumption in the dog (Unverferth et al., 1984; Anderson et al., 1988) and induced coronary vasodilatation (Anderson et al., 1993; Accili et al., 1995) in a time- and concentration-dependent manner (Suzuki et al., 1995). Recent research has shown that VIP microinjection at different locations has different physiological effects on mean arterial blood pressure (Shido et al., 1989; Krowicki et al., 1997). In the heart, the stimulation of VIP receptors (Chatelain, 1984) increased heart rate whereas systemic administration decreases in the mean arterial blood pressure (Rigel, 1988; Roossien et al., 1997). The direct chronotropic effect of VIP in the isolated neonatal canine hearts is to reduce sinus cycle length and AV nodal conduction time (Shvilkin et al., 1988; Pickoff et al., 1994). With conscious sheep, VIP (0.07-1.8 nmol/min) infused into the carotid artery over a 10 min period produced a dose-dependent increase in heart rate. At the highest doses tested, heart rate increased nearly threefold during the infusion while mean arterial pressure declined by 18.5% (Sawangjaroen et al., 1992), A similar response was seen in ewes by Sawangjaroen and Curlewis (1994). The increased heart rate by exogenous VIP could be a result of direct effects on the heart as well as reflex neural activation of the heart in response to peripheral vasodilation. However, the cardiovascular effects of VIP are complex and not clearly understood.

Although VIP acts as a potent bronchodilator (Tang et al., 1995) and double fluorescence immunohistochemistry indicated that VIP was present in high concentrations in normal lung (Bernhard et al., 1989; Nohr et al., 1993; Tang et al., 1995), there is no available data to indicate the effect of VIP on the respiration.

Heart rate and respiratory rate have been used as physiological indicators when animals are stressed by various factors. The heart rate response to stressors is generally accompanied by changes in metabolism. There is a strong positive relationship between heart rate and metabolic rate (Webster, 1983). Therefore, an increase of heart rate induced by VIP

could also be accompanied by a coincidental increase of metabolic rate.

In view of the effects of VIP to increase feed intake and digesta passage in certain circumstances, it is hypothesized that VIP infusion will increase the metabolic rate of sheep. Further, it is hypothesized that the effect of VIP on heat production and heart rate will be modified by the environment in which the animal is housed.

The objectives of this study were to investigate the following questions: 1) does VIP affect metabolic rate, and 2) is there a possible interaction of VIP and thermal environments on metabolic rate and heart rate, and 3) does the route of VIP infusion via gastric artery or jugular vein affect heart rate and respiratory rate differently?

B. Experimental Procedures

Animals and preparation

Sixteen sheep with body weights 40-50 kg were involved in four experiments. A diet of alfalfa pellets (estimated ME 9.4 MJ kg^{-1}, CP 13.4% of DM) was used in all experiments and given to the animals at 9:00 AM. All sheep had free access to water and mineralized salt blocks.

Experiment 1: Four wethers were housed at a temperature of 21 ± 2 °C and fed for 8 h per day *ad libitum*. All sheep were surgically cannulated with a left gastric-artery polypropylene catheter (Okine et al., 1995). During the treatment periods, VIP (1 nmol ml^{-1}) (purity 99.9%, Products No. V3628, Sigma Chemical Co., St. Louis, Ottawa, ON) or saline solution was consecutively infused (1 ml min^{-1}) through the gastric-artery catheter for 10 min of each hour for 3 hours per day for 4 days in a single cross-over design. Infusion started at 9:00 AM, 2 min before feeding.

Heat production (HP) was measured for a total of 5 h duration prior to, during and after VIP or saline infusion in treatment periods. Estimation of heat production was based on the measurements of respired gas and the volume of oxygen consumption by using an open-circuit respiratory apparatus (Young et al., 1975) connected to a respiratory hood. The ventilation rate of the hood was read from a flowmeter (Romometer, Fisher and Porter, Warminster, PA), through which air was drawn at rate of 60-70 L/min, corrected for standard

temperature and pressure. The difference in oxygen concentration between the incoming and outgoing air was obtained from a single-channel paramagnetic oxygen analyzer (Servomex 540A, Sussex, England). The measuring system was calibrated with nitrogen as zero gas and by the procedure of Young et al. (1984). Heat production was calculated using the equation of McLean (1972). The metabolic hood allowed the animal access to water and feed freely during the recording period.

The rectal temperature (Tr) was measured with a telethermometer and thermister probe (Model 46 TUC YSI) at 2:00 pm each day of the treatment periods.

Experiment 2: Four sheep from experiment 1 were offered feed *ad libitum* for 24 h per day and were housed in a cold environment (1 ± 2 °C). Animal preparation, surgery procedures and cannulation were the same as described for experiment 1. During treatment periods, VIP (1 nmol min^{-1}) or saline solution (control) was infused through the gastric artery for 10 minutes each hour for 3 hour on each of 4 consecutive days starting at 9:00 am, 2 minutes before feeding in a single cross-over design.

Metabolic rates and rectal temperatures were measured prior to, during and after infusion treatments as described for experiment 1.

Experiment 3: A complete two factorial cross-over experimental design used four sheep. Animals were offered the diet for 24 h per day and housed first in a thermoneutral environment (21 ± 2 °C) and then in a cold environment (1 ± 2 °C). The preparation and catheterization procedures of the animals were the same as described for experiment 1. During treatment periods, VIP (1 nmol min^{-1}) or saline solution (control) was infused through the gastric artery for 10 minutes each hour for 3 hour on each of 4 consecutive days starting at 9:00 am, 2 minutes before feeding.

Metabolic rates and rectal temperatures were measured prior to, during and after infusion treatments as described for experiment 1.

Experiment 4: Eight sheep in experiment 4 were housed in a warm environment (21 ± 2 °C) and fed for 24 h *ad libitum*. Animal preparation, surgery procedure and catheterization were the same as described for experiment 1. During measurement of heart rate and respiratory rate, VIP (1 or 3 nmol ml^{-1}) or saline solution was infused (1 ml min^{-1}) was infused through either the gastric artery catheter or jugular vein catheter according to

random design such that each sheep received each combination for three 10 min infusion periods. The heart rate (HR) and respiratory rate (RR) were recorded prior to (none), during and after VIP infusion during treatment periods. HR was monitored by three electric probes (one attached to the back and the other two to the fore flanks). ECG signals were recorded by a Beckman physiological recorder (Beckman Model R511A, Schiller Park, Illinois) and expressed by beats per minute (bpm). RR was measured by a elastic tubing tied around the sheep trunk at the last rib. The one end of the tubing was sealed and the other end of the tubing was connected to a pressure transducer (Gould Statham, Oxnard, California) through a Teflon tubing, which in turn was connected to Beckman physiological recorder (Beckman Model R511A, Schiller Park, Illinois).

Statistical Analysis

Heat production and rectal temperature from each of experiments were analyzed by GLM procedure of SAS (1996). The model for experiment 1 and 2 included animals and infusion treatment. The model for experiment 3 included animals, infusion treatments, environmental temperatures and their interactions. Comparison of means was done by the Pdiff option of SAS procedure. Heat production during 10 min infusion periods was also calculated to compare with average data from 15 min prior to and after infusion treatment in experiment 3. Data was analyzed by GLM procedure of SAS with the Pdiff option to do comparison of means. Heart rate and respiratory rate in experiment 4 were measured over the 10 min of each infusion period. Each infusion period produced one replication for each parameter. Data included periods prior to, during and after each infusion treatment. The general Linear Model included animals, infusion treatment dosage level, infusion route and their interactions (SAS, 1996). Comparison of means was done by the Pdiff option in the procedure of least square mean of SAS.

C. Results

Experiment 1

The results of experiment 1 are given in tables 5-1 and 5-2. The metabolizable energy intake was higher in the VIP treatment. Vasoactive intestinal polypeptide infusion did not

influence HP (Table 5-1) or rectal temperature (Table 5-2), compared to saline infusion. Calculated energy retention was improved by VIP treatment by 16.9%.

Experiment 2

Metabolizable energy intake was lower in experiment 2 than for experiment 1. Vasoactive intestinal polypeptide infusion significantly ($P < 0.01$) increased HP (Table 5-1), compared to that of saline infusion. However, rectal temperature was not influenced by the treatment (Table 5-2). As VIP did not affect ME intake in the experiment, the calculated energy retention was 15.8% lower ($P=0.17$) in VIP treatment (Table 5-1).

Experiment 3

Vasoactive intestinal polypeptide infusion did not influence ME intake (Table 5-1), HP (Table 5-1) or rectal temperature (Table 5-2), compared to that of saline infusion in either the warm or cold environment. Cold exposure stimulated a significantly higher ME intake and HP ($P < 0.05$) (Table 5-1) with a significantly lower rectal temperature ($P < 0.01$), compared to that resulting from the warm environment (Table 5-2). There was no interaction of infusion treatment and environmental temperature on metabolic heat production (Figure 5-1) or rectal temperature. In addition, the HP values averaged over 20 min periods prior to, and after VIP infusion in each environment did not show any difference from the HP values averaged over 10 min periods during VIP infusion (Figure 5-2). The results indicated that VIP infusion for a short period did not affect metabolic rate in either the 1 or 22 °C environments for sheep. There was no effect of VIP on calculated energy retention (Table 5-1).

Experiment 4

HR acceleration was noted about 1.5 ± 0.2 min after starting VIP infusion. During most of the individual infusion trials, the increased HR declined 1.5-2.5 min after the end of VIP infusion. However, there were 5 of 48 cases where HR returned to the basal level before VIP infusion was stopped.

Vasoactive intestinal polypeptide infusion via jugular vein at both levels accelerated

HR ($P < 0.05$), compared to no treatment and saline infusion (Table 5-3). VIP infusion via gastric artery caused a concentration-dependent increase of HR. Infusing VIP through the gastric artery at 1 nmol min^{-1} produced a 13.0% higher HR than the saline infusion (121.3 vs 107.4 bpm), but the effect was not statistically significant (Table 5-3). Vasoactive intestinal polypeptide infusion via the gastric artery at 3 nmol/min increased HR to 165.1 bpm ($P < 0.01$) which was the same response as that due to VIP infusion (1 or 3 nmol min^{-1}) through the jugular vein ($P < 0.01$).

VIP infusion through either the jugular vein catheter or gastric artery catheter at a rate of 1 or 3 nmol/min did not alter respiratory rate (Table 5-3). However, a 12.4 % reduction of RR might indicate an inhibitory role of VIP when VIP was infused through the jugular vein catheter at rate of 3 nmol/min.

D. Discussion

HP is an index of internal organic matter metabolism. Except for transient periods of anaerobic metabolism, heat production can be estimated from consumption of oxygen and carbon dioxide production. In this study, oxygen consumption was indirectly used to estimate heat production.

Vasoactive intestinal polypeptide infusion via gastric artery significantly increased HP in the cold environment during experiment 2 (Table 5-1). However, it did not affect HP in the warm environment of experiment 1 or average HP in either cold or warm environments during experiment 3. The reason for these different responses is not clear. In experiment 2, it is possible that VIP increased heat loss in the cold by inducing vasodilation and that the increase in heat production balanced the extra heat loss, since rectal temperature remained the same in both treatments. This response, of course, was not duplicated during experiment 3, where total HP in the cold was less than that in experiment 2 and where rectal temperature tended to decline. HP was higher in the cold than in the warm, similar to previous reports in cattle (McGuire et al., 1989; Miaron and Christopherson, 1995). A 23.8% increase of HP in the cold provides an indication of the relative changes in energy expenditure imposed on the sheep at the two environmental temperatures. During the initial period of exposure to the cold environment, all shorn sheep showed visible shivering, which contributed to the

increases of metabolic rate. The increased HP helps to limit downward deviations of body temperature during cold exposure. There was a reduction in visible shivering of the sheep after the third week of exposure to 1 °C. This may indicate acclimation in agreement with the results of Young (1975). The elevated heat production with reduced visible shivering after 3 wk of exposure to cold could be regard as evidence that the animals had adapted gradually and metabolically to 1 °C. Therefore, the elevated HP by the cold-acclimated and shorn sheep might be attributed, in part, to increased non-shivering thermogenesis. However, the irregular visible shivering and arched back indicate that shivering and increased muscle tone persisted and continued to contribute to increased HP even after 3 weeks in present study. The increased gastric EMG activity in the cold (Chapter 2) provides additional indirect evidence that prolonged exposure to the 1 °C environment for shorn sheep resulted in an increase in metabolic rate to maintain homeothermy.

The cold-exposed animals significantly reduced rectal temperature, compared to thermoneutral-exposed animals (Table 5-2), consistent with observations in a previous study (Christensen. et al., 1991). Christensen et al. (1991) reported that two hour of cold exposure reduced mean rectal temperature from 39.6 to 38.9 in sheep. The reduced rectal temperature indicated tolerance adaptation had occurred (Bruck et al., 1976). Slee (1972) found that the decrease of rectal temperature in response to moderate cold exposure grew larger with repeated exposures, and that low core body temperature was associated with only minor increases in metabolic rate.

Different responses of HR to VIP infusion at 1 nmol/min via different route of administration indicated that the liver plays a role in clearance of VIP from the circulation. On the other hand, VIP infusion through the jugular vein may also reduce its effect on gastrointestinal motility because the lung is another major site for clearance of VIP in the circulation.

The increased heart rate induced by exogenous VIP could be an integrated response involving direct action on the heart (Chatelain et al., 1984; Shvilkin et al., 1988; Rigel, 1988; Pickoff et al., 1994) and peripheral vasodilatation which leads to cardioaccelleration via baroreceptor reflexes (Lundberg et al., 1985; Tang et al., 1995; Krowicki et al., 1997). Chatelain et al. (1984) reported that there are "VIP-preferring" receptors in dog and monkey

heart. The direct chronotropic effect of VIP includes decreases in the spontaneous cycle length, sinus cycle length and AV nodal conduction time (Pickoff et al., 1994). Increases in heart rate due to VIP were reduced significantly by the VIP antagonist [D-p-Cl-Phe6, Leu17] VIP, which alone had no effect on sinus rate (Shvilkin et al., 1988). In one experiment with conscious sheep, VIP was infused into the carotid artery over a 10 min period. There was a dose-dependent increase in heart rate. At the highest doses tested, heart rate increased nearly threefold during the infusion (Sawangjaroen et al., 1992; Sawangjaroen and Curlewis, 1994). In the peripheral circulation, VIP decreased the mean arterial blood pressure in dogs (Rigel, 1988; Roossien et al., 1997) and in sheep (Sawangjaroen et al., 1992), and modulated vasodilation in the cardiovascular system, such as non-cholinergic rectal vasodilatation and coronary vasodilation (Said and Mutt, 1970; Anderson et al., 1983, 1988, 1993; Accili et al., 1995). Blood flow increased significantly to the esophagus, pancreas, atria, and ventricles and to the endocardial and epicardial regions of the left ventricular free wall (Unverferth et al., 1984), and to the pancreaticoduodenal artery (Thulin, 1973). VIP also increased blood flow in the superior mesenteric artery, gastroduodenal artery, portal vein and pancreatic tissue (Inoue et al., 1988). The peripheral vasodilation in these tissues apparently stimulated a reflex increase of heart rate.

Suzuki et al. (1995) reported that VIP induced significant, time- and concentration-dependent vasodilation increases from baseline values (Lundberg et al., 1985). Recent research has shown that VIP microinjection at different locations has different physiological effect. Microinjection of VIP into the dorsal vagal complex in rats (Krowicki et al., 1997) and via the intracerebroventricular route in rats (Shido et al., 1989) increased arterial blood pressure. Mean arterial blood pressure increased in response to VIP in the nucleus raphe obscurus at only the highest dose of 100 pmol, but no changes were observed after microinjection of VIP into the region of the nucleus ambiguus (Krowicki et al., 1997). These local differences of response to VIP may be due to different densities of VIP receptors and different clearance rates of VIP in the specific tissues or organs. Variation in the capacity of the liver and lung for clearing VIP (Luis et al., 1988; Bernhard et al., 1989) might have contributed to the different responses of heart rate to VIP infusion via the jugular vein and gastric artery in present study.

There is a positive correlation of HR and HP (Holmes et al., 1976). Cold exposure stimulates higher HR (McBride and Christopherson, 1984; Miaron and Christopherson, 1995). The increase heart rate partly contributes to higher oxygen consumption and HP (Christensen et al., 1990; Kuhnen and Jessen, 1990). Even if the present study has not measured the HR and HP simultaneously, VIP accelerating HR should potentially induce a higher HP. Clearance by the liver of VIP infused through the gastric artery at 1 nmol/min may have reduced the opportunity for VIP to stimulates HR. This may account for the lack of a consistent HP response to VIP in the present study.

A relatively high density of VIP receptors exist in the lung (Bernhard et al., 1989). Therefore, some functional role of VIP in the lung is possible. Failure of VIP to stimulate a change of respiratory rate in the present study may suggest that VIP has a limited role in regulation of respiratory reflexes. VIP probably regulates physiological functions other than respiratory rate: for example it may influence brochodilation in the lung of sheep.

It is concluded that VIP has little or no direct effect on heat production of sheep except when the animals have already shown a substantial metabolic response to cold exposure. In these circumstances it is possible that VIP increases heat loss due to vasodilation, in which case the animal further increases HP to maintain core temperature.

Table 5-1. Effect of VIP on energy balance [z] (KJ/kg/d).

Experiment	Environment	Item	Infusion		SE	P
			VIP	Saline		
1	Warm	OM intake (g/kg/d)	62.92	55.83	2.82	0.126
		ME intake *	650.28	576.99	29.16	0.126
		Heat Production	181.66	176.26	8.56	0.671
		NE retention	468.63	400.74	27.44	0.131
2	Cold	OM intake (g/kg/d)	42.04	40.73	3.14	0.777
		ME intake	434.53	420.91	32.53	0.777
		Heat production	279.76	241.66	5.84	0.009
		NE retention	154.77	179.24	28.93	0.171
3	Warm	OM intake (g/kg/d)	32.93 [y]	32.37 [y]	1.74	0.925
		ME intake	340.27 [y]	334.51 [y]	17.98	0.925
		Heat production	164.53 [y]	163.34 [y]	8.58	0.977
		NE retention	175.74	171.17	17.54	0.781
	Cold	OM intake (g/kg/d)	39.13 [x]	38.22 [x]	1.74	0.925
		ME intake	404.37 [x]	394.96 [x]	17.98	0.925
		Heat production	202.72 [x]	208.53 [x]	8.58	0.711
		NE retention	201.64	186.43	17.54	0.781
	Average	OM intake (g/kg/d)	36.03	35.29	1.23	0.925
		ME intake	372.32	364.74	12.71	0.925
		Heat production	183.62	185.93	6.07	0.711
		NE retention	188.70	178.80	12.40	0.781

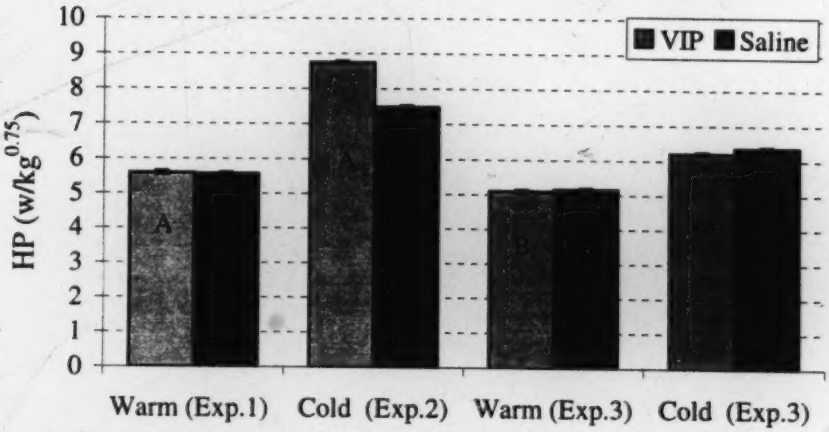
Notes: [z] - the value estimated by the difference of ME intake and heat production.

* ME intake was estimated by 4474.7 (cal/g of OM, measured GE) * 0.673 (apparent OM digestibility, Kennedy, 1985) * 0.82 * OM intake (g/kg/d).

a, b - means with different superscripts in the same row were different ($P<0.01$).

x, y - means with different superscripts on the same item in the same column were different ($P<0.05$).

Figure 5-1. Heat production response to combination of infusion treatments and thermal environments.



A, B - means within an experiment with the different capital letters differ significantly ($P < 0.001$).

Figure 5-2. Heat production (HP) prior to (20 min), during (10 min) and after (20 min) VIP and saline infusions in experiment 3.

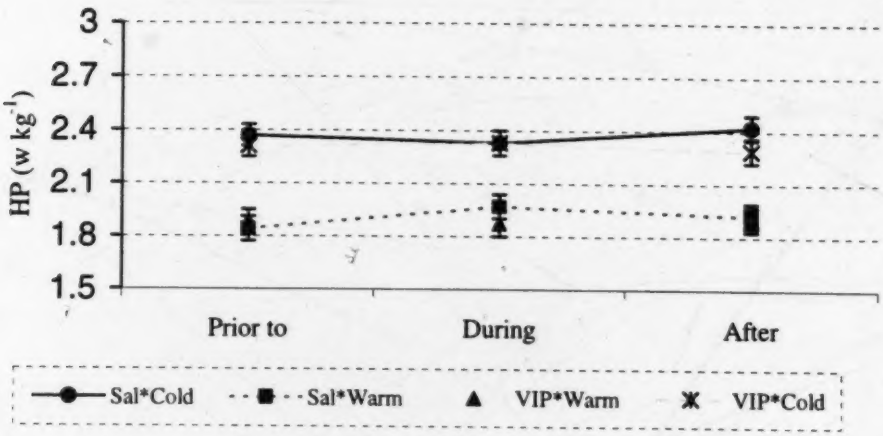


Table 5-2. Rectal temperature (°C) of sheep infused with VIP (1 nmol/min) or saline

Experiment No.	Thermal Environment	Treatment		SE	P
		VIP	Saline		
1	Warm	39.66	39.82	0.07	0.11
2	Cold	39.20	39.20	0.05	1.00
	Warm	39.61 ^{x}	39.69 ^{x}	0.07	0.86
3	Cold	39.29 ^{y}	39.18 ^{y}	0.07	0.86
	Average	39.45	39.43	0.05	0.86

Note: each mean value in the table is average of sixteen observations.

x, y means with different superscripts in the same column were different ($P < 0.001$).

TABLE 5-3. Interaction of route of infusion and infusion treatments on heart rate (beats min^{-1}) and respiration rate (breaths min^{-1}) of sheep in experiment 4.

Item	Route	Infusion treatments (ml/min)				SE	P
		None+	Saline	VIP(1nmol/min)	VIP(3 nmol/min)		
Heart rate	JV*	112.9^{by}	107.8^{by}	157.3^{ax}	169.7^{ax}	5.85	0.001
	GA*	116.7^{by}	107.4^{by}	121.4^{by}	165.1^{ax}	8.28	0.001
	Average	114.8^{c}	107.6^{c}	139.3^{b}	167.4^{a}	5.07	0.001
Respiration rate	JV	80.7	87.3	87.6	76.5	8.03	0.521
	GA	116.8	100.6	109.3	98.9	11.35	0.521
	Average	98.7	94.0	98.4	87.7	6.95	0.648

Notes: Each mean value in the table is average of twenty four observations.

+ - reading prior to saline and VIP infusion treatments.

* JV -- jugular vein catheter; GA -- gastric-artery catheter; Both -- average of JV and GA.

a, b, c -- in columns, means with different superscripts on the same item are different ($\alpha = 0.001$).

x, y -- interaction of infusion routes and treatments, means in rows with different superscripts differ at $\alpha = 0.02$.

E. Literature Cited

Accili, E. A., A. M. Buchan, Y. N. Kwok, J. R. Ledsome, and J. C. Brown. 1995. Presence and actions of vasoactive intestinal peptide in the isolated rabbit heart. Can. J. Physiol. & Pharm. 73(1): 134-9.

Achmadi, J., H. Sano, and Y. Terashima. 1994. Metabolic heat production response to feeding and feed intake in heat-exposed sheep given a concentrate or roughage diet. Anim. Sci. Tech. 65(3): 251-257.

Anderson, F. L., A. C. Kralios, B. Reid, and G. R. Hanson. 1993. Release of vasoactive intestinal peptide and neuropeptide Y from canine heart. Amer. J. Physiol. 265(3 Pt 2): H959-65.

Anderson, F. L., A. C. Kralios, R. Hershberger, and M. R. Bristow. 1988. Effect of vasoactive intestinal peptide on myocardial contractility and coronary blood flow in the dog: comparison with isoproterenol and forskolin. Journal of Cardiovascular Pharmacology. 12(3): 365-71.

Andersson, P. O., S. R. Bloom, A. V. Edwards, J. Jarhult, and S. Mellander. 1983. Neural vasodilator control in the rectum of the cat and its possible mediation by vasoactive intestinal polypeptide. J. Physiol. 344: 49-67.

Bernhard, W., E. Bothe-Sandtfort, H. Koop, W. Cassel, and P. Von Wichert. 1989. Degradation of vasoactive intestinal peptide by isolated, ventilated, and perfused rat lungs. European Journal of Clinical Investigation. 19(6): 506-13.

Blank, M. A., K. Kimura, and B. M. Jaffe. 1988. Regulatory mechanisms in the luminal and portal release of vasoactive intestinal polypeptide during vagal nerve stimulation in the cat. Regulatory Peptides. 23(1): 15-26.

Bruck, K., E. Baum, and H. P. Schwennicke. 1976. Cold-adaptive modifications in man induced by repeated short-term cold exposures and during a 10-day and -night cold exposure. Pflugers Arch. 363: 125-133.

Bunting, L. D. L. S. Sticker, and P. J. Wozniak. 1992. Effect of ruminal escape protein and fat on nitrogen utilization in lambs exposed to elevated ambient temperatures. J. Anim. Sci. 70(5): 1518-25.

Chakder, S., and S. Rattan. 1995. Distribution of VIP binding sites in opossum internal anal

sphincter circular smooth muscle. Journal of Pharmacology & Experimental Therapeutics. 272(1): 385-91.

Chappel, R. W., and R. J. Hudson. 1978. Winter bioenergetics of Rocky Mountain bighorn sheep. Canadian Journal of Zoology. 56 (11): 2388-2393.

Chatelain, P., P. Robberecht, M. Waelbroeck, P. De Neef, J. C. Camus, A. N. Huu, J. Roba, and J. Christophe. 1984. Topographical distribution of the secretin- and VIP-stimulated adenylate cyclase system in the heart of five animal species. Pflugers Archiv - European Journal of Physiology. 397(2): 100-5.

Chevendra, V., and L. C. Weaver. 1992. Distributions of neuropeptide Y, vasoactive intestinal peptide and somatostatin in populations of postganglionic neurons innervating the rat kidney, spleen and intestine. Neuroscience. 50(3): 727-43.

Christensen, R. A., J. J. Kennelly, and R. J. Christopherson. 1991. Gastrointestinal motility and plasma hormone and metabolite response to acute cold stress and somatostatin-14 in sheep. Can. J. Anim. Sci. 71: 1079-1086.

Christensen, R. A., R. J. Christopherson, and J. J. Kennelly. 1990. Effect of somatostatin and chronic cold exposure on hormonal and metabolite concentration, metabolic rate, thermoregulation, and gut motility in sheep. Can. J. Anim. Sci. 70:1073-1083.

Delfino, J. G., and G. W. Mathison. 1991. Effects of cold environment and intake level on the energetic efficiency of feedlot steers. J. Anim. Sci. 69: 4577.

Fahrenkrug, J. 1989. VIP and autonomic neurotransmission. [Review] Pharmacology & Therapeutics. 41(3): 515-534.

Holmes, C. W., D. B. Stephens, and J. N. Toner. 1976. Heart rate as a possible indicator of the energy metabolism of calves kept out of doors. Livest. Prod. Sci. 3(4): 333.341.

Horton, G. M. 1981. Responses of shorn and full-fleeced lambs given two levels of feed intake and exposed to warm and cold temperatures. Am. J. Vet. Res. 42(12): 2151-4.

Inoue, K., S. Sumi, R. Doi, M. Yun, H. Kaji, S. Higashide, H. Minote, Y. Gu, M. Kogire, R. Hosotani, and T. Tobe. 1988. Effect of VIP on splanchnic circulation in dogs. Biomedical Research. 9 (Suppl. 3): 125-129.

Kennedy, P. M. 1985. Influences of cold exposure on digestion of organic matter, rates of passage of digesta in the gastrointestinal tract, and feeding and rumination behaviour

in sheep given four forage diets in the chopped, or ground and pellected form. Br. J. Nutr. 53: 159-173.

Krowicki, Z. K., N. A. Nathan, and P. J. Hornby. 1997. Opposing effects of vasoactive intestinal polypeptide on gastric motor function in the dorsal vagal complex and the nucleus raphe obscurus of the rat. Journal of Pharmacology & Experimental Therapeutics. 282(1): 14-22.

Kuhnen, G., and C. Jessen. 1990. Repeated exposures to cold and the relationship between skin and core temperatures in control of metabolic rate in the goat (*Capra Hircus*). Comp. Biochem. Physiol. 96A: 245-252.

Lam, K. S. 1991. Vasoactive intestinal peptide in the hypothalamus and pituitary. [Review] Neuroendocrinology. 53 (Suppl 1): 45-51.

Llamas-Lamas, G., and D. K. Combs. 1990. Effects of environmental temperature and ammoniation on utilization of straw by sheep. J. Anim. Sci. 68(6): 1719-25.

Luis, J., J. M. Martin, A. el Battari, J. Marvaldi, and J. Pichon. 1988. The vasoactive intestinal peptide (VIP) receptor: recent data and hypothesis. [Review] Biochimie. 70(10): 1311-22.

Lundberg, J. M., A. Anggard, J. Pernow, and T. Hokfelt. 1985. Neuropeptide Y-, substance P- and VIP-immunoreactive nerves in cat spleen in relation to autonomic vascular and volume control. Cell & Tissue Research. 239(1): 9-18.

McBride, G. E., and R. J. Christopherson. 1984. Effects of cold exposure on blood flow to the mammary gland and tissues of the hind limb of the lactating ewe. Can. J. Anim. Sci. 64: 391-402.

McCulloch, J., and L. Edvinsson. 1980. Cerebral circulatory and metabolic effects of vasoactive intestinal polypeptide. Am. J. Physiol. 238(4): H449-56.

McGuire, M. A., D. K. Beede, M. A. DeLorenzo. C. J. Wilcox, G. B. Huntington, C. K. Reynolds, and R. J. Collier. 1989. Effects of thermal stress and level of feed intake on portal plasma flow and net fluxes of metabolites in lactating Holstein cows. J. Anim. Sci. 67(4): 1050-60.

McLean, J. A. 1972. On the calculation of heat production from open-circuit calorimetric measurements. Brit. J. Nutr. 27(3): 597-600.

Miaron, J. O. O., R. J. Chistopherson, R. T. Hardin, R. Mosenthin, and S. Cosgrove. 1995. The effect of α_2-adrenoceptor stimulation with guanfacin on heat production of restricted fed steers kept at -9, 11 or 28 °C and on *ad libitum* fed steers acclimatised to -19 and 22 °C. J. Therm. Biol. 20: 291-298.

Molinero, P., J. R. Calvo. R. Goberna, and J. M. Guerrero. 1985. The interaction of vasoactive intestinal peptide (VIP) with isolated bovine thyroid plasma membranes. Biochemical & Biophysical Research Communications. 128(3): 1336-41.

Nohr, D. L., E. Eiden, and E.Weihe. 1993. Coexpression of vasoactive intestinal peptide, calcitonin gene-related peptide and substance P immunoreactivity in parasympathetic neurons of the rhesus monkey lung. Neuroscience Letters. 199(1): 25-8.

Okine, E. K., J. O. Ole Miaron, S. Cosgrove, and R. J. Christopherson. 1995. Vasoactive intestinal polypeptide inhibits electromyographic activity of the reticulo-omasal orifice in meal-fed sheep in warm and cold environment. Can. J. Anim. Sci. 75:333.

Ottesen, B., and J. Fahrenkrug. 1995. Vasoactive intestinal polypeptide and other preprovasoactive intestinal polypeptide-derived peptides in the female and male genital tract: localization, biosynthesis, and functional and clinical significance. [Review]. American Journal of Obstetrics & Gynecology. 172(5): 1615-31.

Pickoff, A. S., S. N. Wang, A. Stolfi, D. Ramage, N. T. Ross-Ascuitto, D. W. Kydon, and R. J. Ascuitto. 1994. Vasoactive intestinal peptide: electrophysiologic activity in the newborn heart. Pediatric Research. 35(2): 244-9.

Rigel, D. F. 1988. Effects of neuropeptides on heart rate in dogs: comparison of VIP, PHI, NPY, CGRP, and NT. Am. J. Physiol. 255(2 Pt 2): H311-7.

Roossien, A., J. R. Brunsting, A. Nijmeijer, J. Zaagsma, and W. G. Zijlstra. 1997. Effects of vasoactive intestinal polypeptide on heart rate in relation to vagal cardioacceleration in conscious dogs. Cardiovascular Research. 33(2): 392-9.

Said, S. I., and V. Mutt. 1970. Polypeptide with broad biological activity: isolation from small intestine. Science, 169, 1217.

SAS. 1996. SAS/STAT® User's Guide (Version 6, 4th Ed.). SAS Inst. Inc., Cary, NC.

Sawangjaroen, K., and J. D. Curlewis. 1994. Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) on prolactin,

luteinizing hormone and growth hormone secretion in the ewe. Journal of Neuroendocrinology. 6(5): 549-55.

Sawangjaroen, K., C. R. Dallemagne, R. B. Cross, and J. D. Curlewis.1992. Effects of pituitary adenylate cyclase activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) on the cardiovascular system in sheep. Peptides. 13(5): 1029-32.

Shido, O., Y. Yoneda, and T. Nagasaka. 1989. Changes in brown adipose tissue metabolism following intraventricular vasoactive intestinal peptide and other gastrointestinal peptides in rats. Japanese Journal of Physiology. 39(3): 359-69.

Shvilkin, A., P. Jr. Danilo, P. Chevalier, F. Chang, I. S. Cohen, and M. R. Rosen. 1988. Vagal release of vasoactive intestinal peptide can promote vagotonic tachycardia in the isolated innervated rat heart. Cardiovascular Research. 28(12): 1769-73.

Simonneaux, V., A. Ouichon, and P. Pevet. 1990. Vasoactive intestinal peptide stimulates melatonin release from perfused pineal glands of rats. J. Neural. Transm. 79: 69-79.

Slee, J. 1972. Habituation and acclimatization of sheep to cold following exposures of varying length and severity. J. Physiol. 227, 51-70.

Suzuki, H., Y. Noda, S. Paul, X. P. Gao, and I. Rubinstein. 1995. Encapsulation of vasoactive intestinal peptide into liposomes: effects on vasodilation in vivo. Life Sciences. 57(15): 1451-7.

Tang, H., A. Welton, and D. Ganea. 1995. Neuropeptide regulation of cytokine expression: effects of VIP and Ro 25-1553. Journal of Interferon & Cytokine Research. 15(11): 993-1003.

Thulin, L. 1973. Effects of gastro-intestinal polypeptide on hepatic bile flow and splanchnic circulation. *Acta Chir. Scand. Suppl.* 441: 1-31.

Unverferth, D. V., T. M. O'Dorisio, W. W. Muir, J. White, M. M. Miller, R. L. Hamlin, and R. D. Magorien. 1984. Effect of vasoactive intestinal polypeptide on the canine cardiovascular system. Journal of Laboratory and Clinical Medicine. 106(5): 542-550.

Wakade, T. D., M. A. Blank, R. K. Malhotra, R. Pourcho, and A. R. Wakade. 1991. The peptide VIP is a neurotransmitter in rat adrenal medulla: physiological role in controlling catecholamine secretion. J. Physiol. 444:349-62.

Webster, A. J. F. 1983. Environmental stress and the physiology, performance and health of ruminants. J. Anim. Sci. 57(6): 1584-1593.

Young, B. A., T. W. Fenton, and J. A. Mclean. 1984. Calibration methods in respiratory calorimetry. Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology. 56(4): 1120-1125.

Young, B. A., B. Kerrigan, and R. J. Christopherson. 1975. A versatile respiratory pattern analyzer for studies of energy metabolism of livestock. Can. J. Anim. Sci. 55:17.

Young, B. A. 1975. Temperature-induced changes in metabolism and body weight of cattle (Bos taurus). Can. J. Physiol. & Pharm. 53(5): 947-53.

Zimmerman, R. P., T. S. Gates, C. R. Mantyh, S. R. Vigna, C. G. Boehmer, and P. W. Mantyh. 1988. Vasoactive intestinal peptide (VIP) receptors in the canine gastrointestinal tract. Peptides. 9(6):1241-53.

Zudenigo, D., and Z. Lačkovic. 1989. Vasoactive intestinal polypeptide: a potential neurotransmitter. Lijecnicki Vjesnik. 111(9-10): 354-9.

CHAPTER SIX

EFFECT OF WATER RESTRICTION AND ENVIRONMENTAL TEMPERATURES ON METABOLIC RATE AND PHYSIOLOGICAL PARAMETERS IN SHEEP

A. Introduction

Changes in the thermal environment induce a variety of physiological responses in animals (Christopherson and Kennedy, 1983). Metabolic rate is increased during cold exposure in sheep (Christensen et al., 1990), and also during the period of heat stress in cattle (Miaron et al., 1995). Therefore, energy requirements of animals are increased in a cold environment and also during acute heat stress (Miaron et al., 1995). However, there is evidence that a portion of increased energy expenditure in response to environment may exceed that which is required to meet the needs for thermoregulation. There is also evidence that water restriction is accompanied by a decrease in metabolic rate, perhaps indicating an energy conservation response (Choshniak et al., 1995)

Important functions of water, which makes up of more than 50% of body weight, include transportation of nutrients and metabolites, loss of heat by evaporation, and homeostasis of the internal environment of the body. Dehydration of animals often occurs in association with exposure to extreme thermal environments, handling and transport to market, water-shortage and diarrhoea (Purohit, 1979; Robertshaw and Dmi'el, 1983; Dmi'el, 1986). Reductions in the degree of tissue hydration and(or) disturbances in water balance create stress situations for the animals. The stress of dehydration may result in barriers to diffusion and also reduce enzymic activities (Beg, 1990). Moderate dehydration in ruminants has been shown to result from deprivation of water for three days during exposure to heat (Macfarlane et al., 1961; Taneja, 1965; Purohit, 1979). The rate of dehydration in the sheep was 87 ml/kg/24 h (Khan and Ghost, 1985). Under grazing management without drinking water, the Marwari sheep almost reached a limit of physiological tolerance by the end of a period of 13 days indicating that water in the feed plus metabolic water could not sustain the animals (Khan and Ghost, 1972). Disturbances of water balance in sheep have been shown to influence body heat balance (Taneja, 1965; Macfarlane, 1972). During water deprivation in warm or hot environments, changes of

rectal temperature did not show clear patterns in these previous studies (Purohit, 1979; El-Nouty and Hassan, 1983; Olsson and Dahlborn, 1989; Abdelatif and Ahmed, 1994). Plasma osmolality increased in sheep and lactating goats during water deprivation (Olsson and Dahlborn, 1989; Dahlborn et al., 1988; Laden et al., 1987; Abdelatif and Ahmed, 1994). The respiratory rate decreased significantly (to about half of the initial value) with complete deprivation of water in sheep (Macfarlane et al., 1961; Schmidt-Nielsen et al., 1967; Purohit, 1979; Abdelatif and Ahmed, 1994) and goats (Olsson and Dahlborn, 1989). However, More (1984) found that watering only every 72 h over a long period (2 years) did not adversely affect electrolyte metabolism in the adult Chola ewe, as judged by serum Na^+, K^+, Mn^+, P^-, Cl^- and HCO_3^- concentrations.

Dehydration under hot conditions reduced plasma (Schaefer et al. 1990) and also induced the secretion increase of aldosterone and cortisol to maintain biofluid balance (El-Nouty et al., 1980; Schneider, 1990). Progressive increases of packed blood cell volume (PCV) and haemoglobin (Hb) concentration were evident with increased time off feed and water. However, rams deprived of feed for 48 h but with water freely available, did not show changes in plasma creatinine (Naqvi and Rai, 1988). Feeding frequency had a minor effect on plasma creatinine concentration and most differences were related to the level of feed intake; for example, dietary restriction increased creatinine in blood serum (Naqvi and Rai, 1988). Moreover, diet composition has also been shown to influence water intake (Osman and Fadlalla, 1974; Abdelatif and Ahmed, 1992). Most studies describing effects of dehydration have been confounded by reduced feed intake induced by water restriction treatments.

Metabolic rate was found to be decreased by more than 20 % in dehydrated goats (Dmi'el, 1986) and camels (Schmidt-Nielsen et al., 1967). A lowered metabolic rate prolongs the period of time that an animal can survive without eating under dehydration and therefore would have survival value. Furthermore, water restriction usually stimulates an endocrine response which controls metabolic activity depending on the thermal environment (Johnson, 1976). It has been suggested that dehydrated sheep decrease metabolic rate as an adaptive mechanism to reduce water needs for thermoregulation. However, in previous studies, water restriction has been confounded with reduced feed intake and it is not clear whether water restriction by itself influences

metabolic rate and other physiological responses independently of changes in food intake. There has been no research on the effects of water restriction on metabolic and physiological responses of sheep during cold exposure when metabolic rate is generally elevated.

The objectives of this study were to determine whether water restriction would reduce metabolic rate independently of feed intake by studying sheep during a fasting state and also to assess possible effects on blood parameters and interactions between water restriction and environmental temperature.

B. Experimental Procedures

Eight adult wether sheep weighing 88-133 kg, were divided into two groups of four randomly to study the effects of either 24.8 ±1.5 °C (warm) or 0.4±1.2 °C (cold) temperatures in controlled chambers with 24 h continuous light. The animals were fed a ration of alfalfa pellets (estimated 8.3 MJ ME/kg, 14.4% CP) at 1.2 x maintenance (79 g $kg^{-0.75}$ d^{-1}) (National Academy of Science-National Research Council, 1984). All sheep were acclimatised for three weeks to their respective environment before experimental sampling and recording started. During the second week of acclimation all sheep were fitted with jugular vein catheters, which were kept filled between samples with heparinized (40 IU/ml) saline (0.9% NaCl). During each four-day experimental period, all sheep had free access to feed and water on the first day. Feed was withheld from all animals starting at 8:00 pm of the first day through to the end of fourth day. In each environment water was withheld from half the animals from day 2 to day 4 (time when water was returned) (restricted group) and half had free access to water (water group). After 10 days of recovery, treatments were reversed for the second experimental period. These experiments were approved by the Faculty Animal Policy and Welfare Committee of the University of Alberta as meeting the guidelines of the Canadian Council on Animal Care.

Heat production (w/kg: watt per kg of body weight) was measured by an open-circuit respiratory apparatus (Young et al., 1975) during the following time periods: 11-6 h prior to, and 13-19, 37-43 and 61-67 h after the onset of fasting. The apparatus was connected to a metabolic hood to which the sheep had previously been accustomed.

Respiratory monitoring periods were between 9:00 and 15:00 h. The ventilation rate of the hood was read from a flowmeter (Rotameter, Fisher and Porter, Warminster, PA) and the difference in oxygen concentration between incoming and outgoing air was obtained from a single-channel paramagnetic oxygen analyser (Servomex 540A, Sussex, England). The measuring system was calibrated by the procedure of Young et al. (1984). HP was calculated using the equation of Mclean (1972).

Blood samples (10 ml) were collected through jugular catheters at 8:00, 15:00 and 22:00 h daily. Blood samples were transferred immediately to plastic tubes containing heparin, gently mixed by inversion, and kept on ice until centrifuged at 4 °C for 15 min at 3000g. Plasma aliquotes were placed into five tubes and frozen at -25 °C until they were analyzed.

Rectal temperature (Tr) was monitored with a telethermometer and thermistor probe (model 46 TUC YSI) daily at about 14:00 h.

Plasma volume was determined from dilution of the marker T-1824 (Evans Blue, Allied Chemical & Dye Corporation, 40 Rector Street, New York 6, N.Y., U.S.A.) (Gortel et al. (1992). Each sheep was injected with 5 ml T-1824 solution (4.997 mg/ml) through a jugular vein catheter at the first day and the fourth day. Blood samples were collected at zero time and at 20 min intervals for 2 h after injecting T-1824. The sample was centrifuged at 4 °C for 15 min at 1600 x g. Plasma was separated and stored at -25 °C until analysis.

Plasma osmolality was assayed by freezing point depression using an Advanced Osmometer 3W (Advanced Instrument Inc., Needham heights. MA). Each sample was measured in duplicate. Plasma creatinine concentration was determined by creatinine kits (Sigma Diagnostics, St. Louis, MO 63178). Packed cell volume (PCV) was assayed by a Cell-DYN 700 haematology analyser (Sequioir-Turner Corp. Mountain Views, CA) (Gortel et al., 1992). Haemoglobin was estimated using OSM2 Hemoximeter (Radiometer A/S, Emdrupyej 72, DK 2400 Copenhagen NV, Denmark).

Plasma cortisol was assayed by Coat-A-Count Cortisol kits (DPC, Los Angeles, CA 90045), which had a sensitivity of 2 µg/L and interassay coefficient of variation of 4.0-6.8%. Plasma aldosterone concentration without extraction was assayed by a specific radio immunoassay using I^{125}-labelled Coat-A-Count Aldosterone Kits (DPC, Diagnostic

Products Corporation, Los Angeles, CA 90045), which had an approximate sensitivity of 16 pg/ml and interassay coefficient of variation of 2.7-8.7%.

Data were analysed by the general linear model (GLM) procedure of Statistical Analysis System Institute Inc. (SAS 1993). The model included temperature(T), animal(A), water restriction(W) and days(D)(Model: $Y=T_i + W_j + TW_{ij} + A_{k(l)} + WA_{jk(l)} + D_l + DT_{il} + DW_{kl} + DTW_{ikl}$ + error). Pdiff option was used to do multiple comparison of the least-square means.

C. Results and Discussion

Heat production

Cold exposure increased heat production (Table 6-1), compared to that in the warm environment ($P < 0.01$), in agreement with previous reports (Christensen et al., 1991; Miaron and Christopherson, 1992). Average HP was 2.26 w/kg and 0.94 w/kg in cold exposure and thermoneutral temperature, respectively. Fasting depressed ($P < 0.05$) HP in the warm environment during all three days of treatment, but only on day one in the cold environment. In the cold, HP tended to increase again by the third fasting day (day 4). Fasting and fasting plus water restriction decreased HP by 18.6 % and 18.8%, respectively, in the cold room, and by 43.4% and 46.2%, respectively, in the warm room. Cold exposure stimulated a more rapid HP rebound. Water restriction did not induce any further decrease of HP below that due to fasting except for a tendency to decrease transiently on day 1 of fasting and continuous depression in fasting plus water restriction on day 2 in the cold. Reducing HP by fasting in the warm was fully consistent with previous reports which showed reduced HP with increasing the watering interval under hot temperature in sheep (Purohit, 1979; Abdelatif and Ahmed, 1994) and goats (Dmi'el, 1986). However, water restriction in these studies reduced feed intake that likely reduced HP. In contrast, More (1984) reported that use of 72 h watering intervals over a long period (2 years) did not adversely alter metabolism in adult Chokla ewes. These differences may result from breed, sex, fleece coat status and ambient temperatures. Although environmental temperature played an important role in stimulation of HP in present study, there was no interaction among fasting, water restriction and environmental temperatures.

According to one definition, "an animal was considered dehydrated when it lost 20% of its body weight" (Maloiy et al., 1978). Animals in the present experiment did not reach true dehydration status by this definition during three full days of water restriction because the maximal losses of body weight were 7% and 11% in the warm and cold environments, respectively, similar to values (9.9%) for the same deprivation period in sheep reported by Cole (1995). The relatively mild degree of dehydration induced may explain why metabolism was not further decreased by water restriction for three days in our experiment. However, there was considerable variation between individuals.

The water requirement is decreased in a cold environment (Winugroho et al., 1987; EL-Nouty et al., 1988) and special mechanisms of water conservation have been used by herbivorous animals during periods of dehydration (Joyce and Blaxter, 1964; Schmidt-Nielsen et al., 1970). One such mechanism is that the degree of water absorption from the digestive tract appears to increase (Goodall and Kay, 1965; Maloiy et al., 1978; Silankove and Tadmor, 1989). Only in the cold environment did fasting and water restriction tend to reduce HP ($p<0.05$) transiently on the first fasting day. This transient suppression of HP was associated with a sustained high level of plasma osmolality during the early period of fasting (Table 6-4). The recovery of metabolic rate on day 2 or/and 3 of fasting in both cold groups may have been related to increases in water absorption from the forestomach to maintain extracellular fluid volume.

Rectal temperature

The cold-exposed sheep, on average, had higher ($P < 0.05$) rectal temperatures (Tr) than animals in the warm environment (Table 6-2), consistent with a previous report by El-Nouty et al. (1988). However, the initial Tr (day 1) was not affected by thermal environments. The fasting treatment decreased rectal temperature to a greater extent ($P < 0.05$) in the warm than in the cold temperature. In an average environmental temperature of 21 °C, Abdalatif and Ahmed (1994) reported that increasing the watering interval (from 24 h to 72 h) significantly reduced Tr with respect to the control in the morning but not in the afternoon (15:00 h). In our experiment, the absence of any effect of water restriction may have been due to an effect of fasting which masked any effect of water restriction.

Plasma Volume

Plasma volume was not affected by any treatment factors (Table 6-3) although the general average of plasma volume in the warm room was 5.7% less than that in cold room. The results indicated that sheep have a strong capacity to maintain plasma volume, consistent with previous studies (Sneddon, 1993) indicating that ruminants can successfully tolerate dehydration. However, this point was argued by study of Cole (1995) that loss of intracellular water was almost four-fold greater than the loss of extracellular fluid volume during a 3 d feed and water deprivation period. Apparently, effects of dehydration on biofluid and physiological function are still not very clear. The present treatments resulted in only 3.4-7.0% loss of plasma volume. These were less than data from Degen and Kam (1992) which showed that rams lost 41.7% plasma volume after deprivation of water for four days. These differences may be due to different methods of assay and calculation, ability of adaptation to dehydration as inherited differences, or due to feeding conditions (Christopherson and Webster, 1972).

Plasma Osmolality

The average values for plasma osmolality are listed in Table 6-4. During the first day of fasting (day 1 of water restriction), plasma osmolality was reduced ($P < 0.05$) except for the water restriction group in the cold environment. As duration of fasting and water restriction increased, plasma osmolality gradually returned to the initial values. This was generally consistent with previous results of Abdelatif and Ahmed (1994). Water restriction did not significantly increase plasma osmolality in the cold environment, however, cold exposure plus water restriction prevented the decrease in osmolality during day one of fasting and for the average of four days in this study. The decreased osmolality might have been induced by fasting because of reduced supply of electrolytes and nutrients from the diet (Silanikove and Tadmor, 1989). Stored water in the ruminoreticulum (Chosniak et al., 1984) may be an essential factor in the maintenance of osmotic homeostasis. On the first fasting day of our experiment, plasma osmolality decreased, apparently in relation to fasting. The increased osmolality after 35

h may have been associated with the mobilization of ruminoreticular electrolytes or evaporation from the respiration system (Silanikove and Tadmor, 1989; Cole, 1995).

Creatinine

The average concentration of plasma creatinine was significantly higher in the warm than that in the cold environment ($P < 0.01$) (Table 6-5). This is consistent with the report that plasma creatinine was increased and sustained during dehydration during hot weather in Yankasa ewes (Igbokwe, 1993) and Merino sheep fed at submaintenance levels (Keenan and Allardyce, 1986). There was no significant difference between fasting and fasting plus water restriction on plasma creatinine concentration. However with increased time of fasting, water restriction resulted in a rapid increase in plasma creatinine ($p<0.05$) at both temperatures. Fasting plus water restriction resulted in significant increases of plasma creatinine after 24 h in both thermal environments, however, fasting without water restriction only increased plasma creatinine in the warm environment after 48 h treatment. On the basis of the daily average within the temperature, plasma creatinine was increased ($p < 0.05$) with time of fasting in the warm, but not in the cold environment (Table 6-5). If a rise in plasma creatinine is taken as an indication of reduced glomerular filtration rate (Keenan and Allardyce, 1986), our result is consistent with the observation of Nawaz and Shah (1984) who reported a decrease in endogenous creatinine clearance in dehydrated sheep. Moreover, Naqvi and Rai (1988) didn't observe a significant change in the blood creatinine during fasting for up to 48 h, a result that is consistent with the result of fasting without water restriction in both thermal environments during the present study.

Haemoglobin

Effects of treatment on blood haemoglobin concentration are shown in Table 6-6. There was a significantly ($P < 0.05$) lower blood haemoglobin concentration in the warm than that in the cold environment on the control day (day 0). Fasting without water restriction had no effect on blood haemoglobin concentration in either environment. Fasting plus water restriction in the warm environment resulted in an increase of blood haemoglobin after 24 h of treatments ($P < 0.05$). The blood haemoglobin values (11.58-

13.22 g dL^{-1}) in the present investigation were higher than values (8-9 g dL^{-1}) reported by (Christopherson and Webster, 1972) for mature ewes. The difference could have been a result of age and nutritional conditions in the two studies. While blood haemoglobin concentration was shown to increase in the short term during feeding when ewes were meal fed (Christopherson and Webster, 1972), there was no consistent effect of progressive time of fasting in the present study.

Packed cell volume (PCV)

Mean PCV did not differ significantly among treatments (Fig. 7-1). There was a tendancy for water restriction to increase PCV levels with fasting on the second and third fasting day in the warm environment in present study, but this effect was not significant. To some degree, it is similar to previous reports that dehydration under heat stress resulted in a significant rise in PCV (EL-Nouty et al., 1980; Igbokwe, 1993). However, temperature does not seem to induce major changes in blood PCV, on the basis of the present study and other published research (EL-Nouty et al., 1980; Igbokwe, 1993). Hossaini-Hilali et al. (1993) reported that haematocrit increased during 48 h starvation in goats, which is not consistent with the present results where there were no significant effects of fasting on blood PCV.

Plasma Hormones

Daily plasma cortisol level was changed with fasting time (Table 6-7). The concentration on the fourth experimental day and control day were significantly greater than those of day 2 and day 3 ($P < 0.05$), in agreement with data from lactating goats (Olsson and Dahlborn, 1989). Plasma cortisol level on the second and third day was 20-70% lower than the control day and 44-84% lower than the fourth day of fasting. The cortisol concentration in the fourth day was 44-106% higher than the control day in both fasting and fasting plus water restriction. The increased level of cortisol was mainly induced by the high level of the (15:00 h) mid-day sample because no effects of treatments on plasma cortisol level were found at 8:00 h or 22:00 h. This pattern was a departure from the finding that cortisol level displays more variation in the morning than in the afternoon (Abdelatif and Ahmed, 1994). The higher level of cortisol at midday in

this study may have been related to a relatively higher physical activity level at this time because most of animals stood from noon to 5:00 PM. Sheep may adapted to daily management at afternoon.

Although plasma cortisol level in the cold was 27% higher than that in the warm, there was no significant effect of temperature ($P = 0.07$) on plasma cortisol level as a result of large variation among animals. This is in agreement with findings by EL-Nouty et al., (1983) in cows, but it is at variance with results from other studies (Yousef and Johnson, 1985; EL-Nouty et al., 1980). El-Nouty et al. (1980) found that dehydration under high temperatures induced a decrease of plasma cortisol level in cattle. Based on the present result, fasting is the main factor to induce cortisol decreases in the first two fasting days, but after a more extended fast, the animal appeared to activate a strong physiological response to increase plasma cortisol level. Water restriction did not produce any additional effect on plasma cortisol level over and above changes related to fasting and also there were no interactions between temperature and water restriction for plasma cortisol. This is similar to the report by Olsson and Dahlborn (1989) who showed that the combination of heat stress and water deprivation did not cause any increase in plasma cortisol level in non-lactating goats. Cortisol level was not affected by 24 h dehydration in Clun Forest sheep (Parrott and Thornton, 1987), but the combination of dehydration and physiological stress did increase cortisol in lactating animals. In desert bred Butana rams fed solely on chopped lucerne hay in the early dry cool season, plasma cortisol levels went down during the second day of water restriction and then increased during the third day of water restriction, similar to the present study (Abdelatif and Ahmed, 1994). However, the results of Abdelatif and Ahmed (1994) were confounded by the interaction of feed and water intake. Studies with different types of sheep suggest that there is breed variation in responses to dehydration (Olsson and Dahlborn, 1989). Based on current results and other studies, sheep appear to adjust to fasting by initially depressing cortisol levels during the first 48 h, but after that, sheep will respond by activation of cortisol response to help adapt to prolonged fasting. On the fourth fasting day in water restricted animals plasma cortisol concentration in the cold environment was higher than those in the warm environment, suggesting a possible link to the higher energy demand in the cold.

Plasma aldosterone was not affected by any of the present treatments (Table 6-8), similar to previous research in dog (Zucker et al., 1982). However, aldosterone concentration rose significantly between 48 and 72 h of an induced dehydration, and were sustained at significantly higher levels in the Namib Horse (Sneddon, 1993). This was believed to coincide with aldosterone-mediated movement of fluid from the gastrointestinal tract to the intravascular space. Unfortunately, the data on plasma aldosterone measured by RIA kits in the present research was highly variable. A substantial number of sheep plasma samples had aldosterone levels, which were below detection limits. This problem of quantifying plasma aldosterone in the sheep has also been reported by Curran-Everett et al. (1988).

Feed and Water Intake

During the experimental period, water intakes in the sheep that were fasted without water restriction differed significantly in the two environments (Table 6-9), even though feed intake was kept the same in all treatments on the control day. During the fasting period sheep treated by fasting without water restriction voluntarily reduced water intake as the development of fasting time in both thermal environments. The reduction of water intake was more rapid in the cold than in the warm environment. The average water intake in the warm was about twice as high as that in the cold. The significantly depressed water intake in response to fasting illustrates the interdependence of dry matter intake and water intake in agreement with previous studies (Macfarlane and Howard, 1972; Silanikove et al., 1987). In one experiment, restriction of water availability to once every 24, 48, or 72 h caused average reductions of 39, 60, and 80 % in the voluntary consumption of feed (Silanikove and Tadmor, 1989). The higher temperature increased water intake of the animal in this study, in agreement with reports by Olsson and Dahlborn (1989) and Macfarlane et al. (1963).

In conclusion, fasting depressed HP significantly and progressively over 3 d in the warm but reduced HP only transiently on day 1 of the fast in the cold environment. Fasting also reduced rectal temperature in both thermal environments but the decrease was transient in the cold environment and reflected the transient change in HP. The decrease in HP during fasting was slightly but not significantly larger during water

restriction. Cold-exposure of animals induced significantly higher HP and rectal temperatures. Thermal exposures and water restriction for three days did not consistently affect plasma volume, haemoglobin and PCV. However, plasma creatinine concentration was increased by the warm temperature and water restriction. Plasma cortisol showed a biphasic response. It was significantly depressed during the first 48 h of fasting but reached its highest values on day three of fasting. The present results suggest that water restriction, per se, for three days resulted in only moderate physiological responses in sheep exposed to temperatures of 0 and 23 °C.

Table 6-1. Heat production (w/kg) response to water restriction and environmental temperatures in sheep.

Day of water	*Cold*		*Warm*	
Restriction	*R*	*W*	*R*	*W*
0	2.491^{ax}	2.491^{ax}	1.404^{bx}	1.404^{ax}
1	1.973^{by}	1.103^{by}	0.855^{by}	0.900^{by}
2	2.128^{ay}	2.215^{axy}	0.670^{by}	0.690^{by}
3	2.305^{axy}	2.355^{axy}	0.733^{by}	0.815^{aby}
Mean	2.232^{a}	2.283^{a}	0.918^{b}	0.964^{b}
Average	2.257^{a}		0.941^{b}	
Pooled SE	0.041			

Notes: a, b - means with the same superscript letter in the row are not different ($P > 0.05$).

x, y - means with the same superscript letter in the same column are not different ($P > 0.05$).

R is the treatment of fasting plus water restriction.

W is the treatment of fasting without water restriction.

Table 6-2. Effect of water restriction and environmental temperatures on rectal temperature (°C) in sheep.

Day of water	*Cold*		*Warm*	
Restriction	*R*	*W*	*R*	*W*
0	39.87 ^{ax}	39.81 ^{ax}	39.83^{ax}	39.83 ^{ax}
1	38.98 ^{az}	38.93 ^{ay}	38.91 ^{ay}	38.80^{ay}
2	39.20 ^{ayz}	39.25 ^{axy}	38.58 ^{by}	38.63 ^{by}
3	39.78 ^{axy}	39.37 ^{abxy}	38.86 ^{by}	38.99 ^{aby}
Mean	39.47 ^{a}	39.34 ^{ab}	39.05 ^{b}	39.05 ^{b}
Average	39.41 ^{a}		39.05 ^{b}	
Pooled SE	0.08			

Notes: each mean value in the table is average of four observations.

a, b - means with the same superscript letter in the row are not different ($P > 0.05$).

x, y - means with the same superscript letter in the same column are not different ($P > 0.05$).

R is the treatment of fasting plus water restriction.

W is the treatment of fasting without water restriction.

Table 6-3. Plasma Volume (L) under different water restriction and environmental temperatures in sheep.

Day of water	*Cold*		*Warm*	
Restriction	*R*	*W*	*R*	*W*
0	4.317	4.396	3.981	4.358
3	4.114	4.154	3.844	4.053
Mean	4.216	4.275	3.912	4.205
Average	4.245		4.059	
Pooled SE	0.257			

Notes: each mean value in the table is average of four observations.

R is the treatment of fasting plus water restriction.

W is the treatment of fasting without water restriction.

Table 6-4. Response of plasma osmolality (mOsmol/kg H_2O) to water restriction and environmental temperatures in sheep.

Day of water	*Cold*		*Warm*	
Restriction	*R*	*W*	*R*	*W*
0	297.5 ^{ax}	297.5 ^{ax}	293.5^{ax}	293.5 ^{ax}
1	293.5 ^{ax}	289.6 ^{ay}	286.1 ^{ay}	286.4^{ay}
2	298.4 ^{ax}	293.4 ^{axy}	292.5 ^{ax}	291.4 ^{axy}
3	299.1 ^{ax}	294.8 ^{abxy}	297.5 ^{abx}	292.4 ^{bxy}
Mean	297.1	292.3	292.4	290.9
Average	294.7		291.7	
Pooled SE	1.33			

Notes: each mean value in the table is average of four observations.

a, b - means with the same superscript letter in the row are not different ($P > 0.05$).

x, y - means with the same superscript letter in the same column are not different ($P > 0.05$).

R is the treatment of fasting plus water restriction.

W is the treatment of fasting without water restriction.

Figure 6-1. Effect of water restriction and environmental temperatures on packed cell volume (%) in sheep. Where: C*W is combination of the cold environmental temperature and fasting without water restriction; C*R combination of cold environmental temperature and fasting with water restriction; W*W combination of warm environmental temperature and fasting without water restriction; W*R combination of warm environmental temperature and fasting with water restriction.

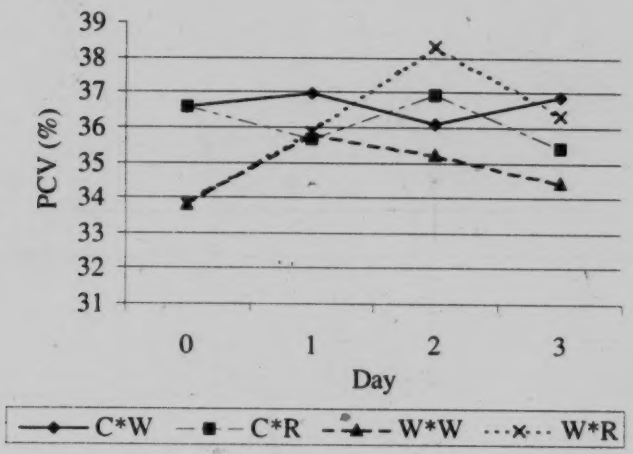


Table 6-5. Plasma creatinine concentration (mg/L) affected by water restriction and environmental temperatures in sheep.

Day of water	*Cold*			*Warm*		
Restriction	*R*	*W*	*Ave*	*R*	*W*	*Ave*
0	5.8 ^{az}	5.8 ^{ax}	5.8 ^{ax}	7.0 ^{ay}	7.0 ^{ay}	7.0 ^{az}
1	6.2 ^{byz}	6.3 ^{bx}	6.3 ^{bx}	8.0 ^{ax}	7.6. ^{aby}	7.8 ^{ayz}
2	6.9 ^{bcx}	6.2 ^{cx}	6.5 ^{bx}	9.5 ^{ax}	8.1 ^{aby}	8.8 ^{ay}
3	6.6 ^{bxy}	6.5 ^{bx}	6.6 ^{bx}	9.4 ^{ax}	10.3 ^{ax}	9.9 ^{ax}
Mean	6.2 ^{b}	6.4 ^{b}	6.3 ^{b}	8.5 ^{a}	8.2 ^{a}	8.4 ^{a}
Average		6.3 ^{b}			8.4 ^{a}	
Pooled SE			0.30			

Notes: each mean value in the table is average of four observations.

a, b - means with the same superscript letter in the row are not different ($P > 0.05$).

x, y - means with the same superscript letter in the same column are not different ($P > 0.05$).

R is the treatment of fasting plus water restriction.

W is the treatment of fasting without water restriction.

Table 6-6. Effects of water restriction and environmental temperatures on blood haemoglobin concentration (g dL^{-1}) in sheep.

Day of water	*Cold*			*Warm*		
Restriction	*R*	*W*	*Ave*	*R*	*W*	*Ave*
0	12.54 ^{axy}	12.54 ^{ax}	12.54 ^{x}	11.58 ^{bz}	11.58 ^{bx}	11.58 ^{y}
1	12.33 ^{axy}	12.65 ^{ax}	12.49 ^{x}	12.14 ^{ayz}	12.15 ^{ax}	12.15 ^{xy}
2	12.81 ^{abx}	12.52 ^{abx}	12.66 ^{x}	13.22 ^{ax}	12.13 ^{bx}	12.70 ^{x}
3	11.87 ^{ay}	12.57 ^{ax}	12.22 ^{x}	12.64 ^{axy}	12.03 ^{ax}	12.34 ^{x}
Mean	12.38	12.57	12.48	12.40	11.97	12.19
Average		12.48			12.18	
Pooled SE			0.21			

Notes: each mean value in the table is average of four observations.

a, b - means with the same superscript letter in the row are not different ($P > 0.05$).

x, y - means with the same superscript letter in the same column are not different ($P > 0.05$).

R is the treatment of fasting plus water restriction.

W is the treatment of fasting without water restriction.

Table 6-7. Effect of water restriction and environmental temperatures on average level of plasma cortisol concentration (μg/L) in sheep.

Day of water	*Cold*		*Warm*	
Restriction	*R*	*W*	*R*	*W*
0	18.2 ^{ay}	18.2 ^{axy}	14.2 ^{axy}	14.2 ^{axy}
1	7.0 ^{ay}	14.5 ^{axy}	7.9 ^{axy}	4.2 ^{ay}
2	7.1 ^{ay}	7.7 ^{ay}	6.1 ^{ay}	5.6 ^{ay}
3	37.4 ^{ax}	26.2 ^{abx}	20.9 ^{bx}	26.5 ^{abx}
Mean	17.4	16.6	12.3	12.6
Average	17.0		12.4	
Pooled SE	1.70			

Notes: each mean value in the table is average of four observations.

a, b - means with the same superscript letter in the row are not different ($P > 0.05$).

x, y - means with the same superscript letter in the same column are not different ($P > 0.05$).

R is the treatment of fasting plus water restriction.

W is the treatment of fasting without water restriction.

Table 6-8. Effect of water restriction and environmental temperatures on plasma aldosterone (ng/L) in sheep.

Day of water	*Warm*		*Cold*	
Restriction	*R*	*W*	*R*	*W*
0	101.6	66.0	67.9	97.1
1	67.3	100.3	121.9	90.4
2	16.2	152.2	77.3	71.8
3	113.1	99.3	21.0	79.0
Mean	74.6	104.5	71.8	84.6
Average	89.6		77.7	
Pooled SE	11.7			

Notes: each mean value in the table is average of four observations.

R is the treatment of fasting plus water restriction.

W is the treatment of fasting without water restriction.

Table 6-9. Effect of fasting on water intake in sheep.

Treatment Day	Cold	Warm
0	2.80 ^{bx}	4.87 ^{ax}
1	0.58 ^{by}	3.48 ^{axy}
2	0.90 ^{ay}	1.55 ^{ayz}
3	0.30 ^{ay}	0.85 ^{az}

Notes: each mean value in the table is average of four observations.

a, b - means with the same superscript letter in the row are not different ($P > 0.05$).

x, y - means with the same superscript letter in the same column are not different ($P > 0.05$).

D. Literature Cited

Abdelatif, A. M., and M. M. M. Ahmed. 1992. Thermoregulation, water balance and plasma constituents in Sudanese desert sheep: responses to diet and solar radiation. J. Arid Environ. 25: 387-395.

Abdelatif, A. M., and M. M. M. Ahmed. 1994. Water restriction, thermoregulation, blood constituents and endocrine responses in Sudanese desert sheep. J. Arid Environ. 26:171-180.

Beg, M. A. A. 1990. Theoretical approach to life processes: Part V. Postulates of the hypothesis on aging. Pakistan Journal of Scientific and Industrial Research 33(3): 92-101

Chosniak, I., C. Wittenberg, J. Rosenfeld, and A. Shkolnik, 1984. Rapid rehydration and kidney function in the blank Bedouin goat. Physiol. Zool. 57:573-579.

Christensen, R. A., R. J. Christopherson, and J. J. Kennedy. 1990. Effect of somatostatin and chronic cold exposure on hormonal and metabolite concentration, metabolic rate, thermoregulation and gut motility in sheep. Can. J. Anim. Sci. 70:1073-1083.

Christensen, R. A., J. J. Kennedy, and R. J. Christopherson. 1991. Gastrointestinal motility and plasma hormone and metabolite response to acute cold stress and somatostatin-14 in sheep. Can. J. Anim. Sci. 71:1079-1081.

Christopherson, R. J., and A. J. F. Webster. 1972. Changes during eating in oxygen consumption, cardiac function and body fluids of sheep. J. Physiol. London. 221 (4): 441-457.

Christopherson, R. J., and P. M. Kennedy. 1983. Effect of thermal environment on digestion in ruminants. Can. J. Anim. Sci. 63:477-496.

Cole, A. N. 1995. Influence of a three-day feed and water deprivation on gut fill, tissue weight and tissue composition in mature wethers. J. Anim. Sci. 73:2548-2557.

Curran-Everett, D. C., J. R. Claybauch, K. Miki, S. K. Hong, and J. A. Krasney. 1988. Hormonal and electrolyte responses of conscious sheep to 96 h of hypoxia. Am. J. Physiol. 255(2pt2): R274-83.

Dahlborn, K., K. Holtenius, and K. Olsson. 1988. Effects of intraruminal loads of saline or water followed by voluntary drinking in the dehydrated lactating goat. Acta Physiological Scandinavia. 132:67-73

Degen, A. A., and M. Kam. 1992. Body mass loss and body fluid shifts during dehydration in Dorper sheep. J. Agric. Sci. 119:410-422.

Dmi'el, R. 1986. Selective sweet secretion and panting modulation in dehydrated goats. J. Therm. Biol. 11(3):157-159.

El-Nouty, F. D., and G. A. Hassan. 1983. Thyroid hormone status and water metabolism in Hereford cows exposed to high ambient temperature and water deprivation. Indian J. Anim. Sci. 53(8):807-812.

El-Nouty, F. D., G. A. Hassan, T. H. Taher, M. A. Samak, Z. Aboelezz, and M. H. Salem. 1988. Water requirements and metabolism in Egyptian Barki and Rahmani sheep and Baladi goats during spring, summer and winter seasons. J. Agric. Sci. Camb. 111:27-34.

El-Nouty, F. D., I. M. Elbanna, T. P. Davis, and H. D. Johnson. 1980. Aldosterone and ADH response to heat and dehydration in cattle. J. Appl. Physiol; Respirat. Environment, Exercise, Physiol. 48(2):249-255.

Goodall, E. D., and R. M. B. Kay. 1965. Digestion and absorption in the large intestine of the sheep. Journal of Physiology, London 176:12-23.

Gortel, K., A. L. Schaefer, B. A. Young, and S. C. Kawamoto. 1992. Effects of transport stress and electrolyte supplementation on body fluids and weights of bulls. Can. J. Anim. Sci. 72: 547-553.

Hossaini-Hilali, J., K. Benlamlih, and K. Dahlborn. 1993. Fluid balance and milk secretion in the fed and feed-deprived black Microoan goat. Small Ruminant research. 12:271-285.

Igbokwe, I. O. 1993. Haemoconcentration in Yankasa sheep exposed to prolonged water deprivation. Small ruminant Research. 12:99-105.

Johnson, H. D. 1976. Effects of heat on endocrine response of domestic and laboratory animals. Vol. 1:pp.27-41. In S.M. Tromp, Ed. Progress in Biometeorology: Amsterdam, Swets and Zeitlinger.

Joyce, J. P. and K. L. Blaxter. 1964. Respiratory in sheep in cold environment. Research in Veterinary Science 5:506-516.

Keenan, D. M., and C. J. Allardyce. 1986. Changes of plasma creatinine levels of sheep during submaintenance feeding. Australian Veterinary Journal. 63:29-30.

Khan, M. S. and P. K. Ghosh. 1985. Body-water relationships during water deprivation in desert animals. Indian J. Anim. Sci. 55(3):169-172.

Laden, S., L. Nehmadi, and R. Yagil. 1987. Dehydration tolerance in Awassi fat-tailed sheep. Can. J. Zool. 65(2):363-367.

Macfarlane, W. V., and B. Howard. 1972. Comparative water and energy economy of wild and domestic mammals. Symposium of the Zoological Society of London. 31:261-296.

Macfarlane, W. V., R. J. H. Morris, and B. Howard. 1963. Turnover and distribution of water in desert camels, sheep, cattle and kangaroos. Nature, London. 197:270-271.

Macfarlane, W. V., R. J. H. Morris, B. Howard, McDonald, and O. E. Budtsolsen. 1961. Water and electrolyte changes in tropical Merino sheep, exposed to dehydration during summer. Aust. J. Agric. Res. 12:889.

Maloiy, G. M. O., C. R. Taylor, and E. T. Clemens. 1978. A comparison of gastrointestinal water content and osmolality in East African herbivores during hydration and dehydration. J. Agric. Sci. Camb. 91:249-254.

Mclean, J. A. 1972. On the calculation of heat production from open-circuit calorimetric measurements. Br. J. Nutr. 27:597-600.

Miaron, J. O. O., and R. J. Christopherson. 1992. Effect of prolonged thermal exposure on heat production, reticular motility, rumen-fluid and -Particular passage rate constants, and apparent digestibility in steers. Can. J. Anim. Sci. 72:809-819.

Miaron, J. O. O., R. J. Chistopherson, R. T. Hardin, R. Mosenthin, and S. Cosgrove. 1995. The effect of α_2-adrenoceptor stimulation with guanfacin on heat production of restricted fed steers kept at -9, 11 or 28 °C and on ad libitum fed steers acclimatised to -19 and 22 °C, J. Therm. Biol. 20:291-298.

More, T. 1984. Effects of long-term intermittent watering on plasma electrolytes in Chokla sheep under semi-arid conditions. Indian J. Anim. Sci. 54(4):391-395.

Naqvi, S. M. K., and A. K. Rai. 1988. Effect of fasting on some biochemical constituents of blood in Avivastra sheep. Indian J. Anim. Sci. 58:1079-1081.

Nawaz, M., and B. H. Shah. 1984. Renal clearance of endogenous creatinine and urea in sheep during summer and winter. Res. Vet. Sci. London: British Veterinary association. 36 (2): 220-224.

National Academy of Sciences-National Research Council. 1984. The nutrient requirements of sheep. NAS-NRC, Washington, D. C.

Olsson, K., and K. Dahlborn. 1989. Fluid balance during heat stress in lactating goats. Quart. J. Exp. Physiol. 74:645-659.

Osman, H. E., and B. Fadlalla, 1974. The effect of level of water intake on some aspects of digestion and nitrogen metabolism of the desert sheep of the Sudan J. Agric. Sci. Camb. 82 (1): 61-69.

Parrott, R. F., and S. N. Thornton. 1987. Effects of psychological stress and dehydration on plasma cortisol, oxytocin and vasopressin in Clun Forest sheep. Physiological Society. 91p.

Purohit, G. F. 1979. Water metabolism in desert sheep breeds some physiological responses of dehydration and rehydration in pure and cross bred desert sheep. Trans. Isdt. & Ucds. 4(2): 84-88.

Robertshaw, D., and R. Dmi'el. 1983. The effect of dehydration on the control of panting and sweating in the black Bedouin goat. Physiol. Zool. 56: 412-418.

Choshniak, I., N. Ben-Kohav, C. R. Taylor, D. Robertshaw, R. J. Barnes, A. Dobson, V. Belkin, and A. Shkolnik. 1995. Meatbolic adaptations for desert survival in the Bedouin goat. American Journal of Physiology. 268(5 Pt 2): R1101-1110.

SAS. 1993. SAS/STAT® User's Guide (Version 6, 6th Ed.). SAS Inst. Inc., Cary, NC.

Schaefer, A. L., S. D. M. Jones, A. K. W. Tong, P. Lepage, and N. L. Murray. 1990. The effects of withholding feed and water on selective blood metabolites in market-weight beef steers. Can. J. Anim. Sci. 70: 1151-1158.

Schmidt-Nielsen, K., E. C. Crawford, A. E. Newsome, K. S. Rawson, and H. T. Hammel. 1967. Metabolic rate of camels: Effect of body temperature and dehydration. Am. J. Physiol. 212:341-346.

Schmidt-Nielsen, K., F. R. Hainsworth, and D. E. Murrish. 1970. Counter-current heat exchange in the respiratory passage: effect on water and heat balance. Respiratory Physiology. 9:263-276.

Schneider, E. G. 1990. In water deprivation osmolality become an important determination of aldosterone secretions. News. Physiol. Sci. 5:197-201.

Silanikove, N., and A. Tadmor. 1989. Rumen volume, saliva flow rate, and systemic fluid homeostasis in dehydrated cattle. Am. J. Physiol. 256:R809-R815.

Silanikove, N., Z. Holzer, D. Cohen, R. Benjamin, M. Gutman, and A. Meltzer. 1987. Interrelationship between metabolism of tritiated water, sodium and dry matter intake in beef cows, fed poultry litter and wheat straw in free choice. Comp. Biochem. Physiol. 88A: 113-118.

Sneddon, J. C. 1993. Physiological effects of hypertonic dehydration on body fluid pools in arid-adapted mammals. How do Arab.-based horse compare. Camp. Biochem. Physiol. 104A:201-213.

Taneja, G. C. 1965. Effect of varying frequency of watering during summer on the rectal temperature, respiration, body weight and packed cell volume of blood of sheep. Indian J. Exp. Biol. 3:259.

Winugroho, M., V. A. Walker, and B. A. Young. 1987. Energy and water kinetics in sheep acclimatised to 0, 20 and 40 °C. Agriculture and Forestry Bulletin, University of Alberta Special Issue, 66th Annual Feeders Day Report, 91-92.

Young, B. A., B. Kerrigan, and R. J. Christopherson. 1975. A versatile respiratory pattern analyzer for studies of energy metabolism of livestock. Can. J. Anim. Sci. 55: 17-22.

Young, B. A., T. W. Fenton, and J. A. Mclean. 1984. Calibration methods in respiratory calorimetry. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 56:1120-1125.

Yousef, M. K., and H. D. Johnson. 1985. Endocrine system and thermal environment. In M. K. Yousef (Ed.): Stress Physiology in Livestock, Vol. 1: P. 133-141. Boca Raton, Fla. CRC Press.

Zucker, A., S. D. Gleason, and E. G. Schneider. 1982. Renal and endocrine response to water deprivation in dog. Am. J. Physiol. 242: R296-R302.

CHAPTER SEVEN

GENERAL DISCUSSION AND CONCLUSIONS

A. Discussion

The growth and performance of animals depends on the availability of substrate that either is mobilized from the body energy stores or is obtained from digestion of ingested feed. Increasing feed ingestion and reducing energy expenditure in modern production systems have been goals in many studies. The efficiency of using the nutrient resources is influenced by genetic potential, physiological status, diet type and environmental factors. In normal healthy animals, feed resources and environmental temperature are dominant factors which affect animal feeding behaviour and utilization through neural reflexes and hormonal regulation. When high quality concentrate feed is supplied that avoids the limiting effects of physical capacity, animals, including ruminants, vary their intakes in inverse relationship to energy density of the food, through feedbacks which reflect metabolites, hormones and substrates. When ruminants are fed roughage diets, on the other hand, physical capacity and digesta passage rates tend to become limiting to voluntary feed intake. In these circumstances, propulsive motility, sphincter action and fluid dynamics can modify the ability of the animal to increase intake to compensate for increased energy demands. Recently, there has been wide interest in the role of gut hormones, including vasoactive intestinal polypeptide (VIP), as a modulator of gastrointestinal motility, secretion and passage rate. Our current results demonstrated that exogenous VIP infusion causes a relaxation of the reticulo-omasal orifice (ROO). This structure is putatively considered as a key structure controlling digesta outflow from the reticulorumen (Bueno, 1972; Ruckebush, 1988) and further to regulate intake. Some researchers also reported that VIP modifies the cardiovascular system, i.e. increasing heart rate and dilating peripheral blood vessel, so that VIP may potentially cause a change of metabolic rate. The present study is the first such project to focus on how VIP influences feed intake and heat production in ruminants. It is known that environmental temperature affects both heat production and feed intake. In a cold

environment, an increase in feed intake could be related to the increased energy demand. However, increased digesta passage rates could also facilitate increase feed intake in a cold environment. The relative importance of each of these factors is not clear. There have been no previous studies on interactions of VIP and environmental temperature on intake and metabolic rate.

The present studies were carried out to test our hypotheses that: 1) VIP relaxes the ROO to facilitate digesta outflow from the reticulo-rumen and to potentially improve intake; 2) VIP regulates the cardiovascular system and hence may modify metabolic rate, 3) moderate exposure to cold temperature may induce intake and metabolic rate changes and may interact with VIP to influence animal performance and affect myo-electrical activity, 4) the changed metabolic rate in sheep exposed to different thermal environments may be diminished by water restriction that would be beyond that produced by only fasting.

VIP infusion induced a higher DM, OM and CP intake in sheep when feed was available for 8 h per day, but not when feed was available for 24 h per day. These results on feed intake support our hypothesis that inhibition of ROO activity by VIP, increases opening of the ROO after the second phase of reticular biphasic contraction to allow increased digesta passage. This facilitated digesta outflow from the reticulorumen, in turn, may stimulate an increase of feed intake. However, upward regulation of digesta flow rate at the proximal duodenum and terminal ileum was not significantly affected by VIP treatment when specific treatment means were statistically compared within individual experiments. In this study, VIP was infused intermittently for only 30 -60 min each day. These relatively short infusion periods (30 min in 3 h) may have reduced the probability of exerting a significant effect on duodenal passage over the entire 24 h period per day. A similar argument might account for the lack of effect of VIP on intake of sheep with 24 h access to feed. The increased infusion frequency and dosage of VIP contributed to the bigger variance of intake and a tendency to increase individual AA supply to the duodenum. The relatively small number of animals and significant animal to animal variance of response to VIP treatment may also explain the lack of significant increases of digesta and nutrient flows in response to VIP because the affinity of VIP receptors or subtype receptors on smooth muscle of the forestomach to exogenous

VIP may vary among individual animal. Other sources of variation may have included possible error in obtaining representative digesta samples from the T-type cannulae, which could have reduced accuracy in quantifying digesta flow (Harmon and Richards, 1997).

Within the individual experiments the significant effects of VIP to increase food intake were accompanied by relatively small and non-significant changes in DM, OM or AA passage to the small intestine. The substantially larger intake responses to cold exposure and 24 h access to feed, on the other hand, were accompanied by significant increases in passage of OM and AA to the duodenum. When the data from all experiments were pooled, regression analysis demonstrated strong positive relationships ($P < 0.001$) between intake and duodenal passage of DM and OM and AA (Figure 8-1). While these positive relationships do not prove "cause and effect", they do suggest that factors which increase intake of a pelleted forage diet will ultimately result in delivery of more total nutrients, including amino acids, to the small intestine. Therefore, it is likely that the VIP-induced increase in intake among animals on 8 h time restricted access to feed will be accompanied by improved intestinal supply of nutrients. This is of biological importance and may improve the nutritional status of the animals. This illustrates that duodenal flow and intake are highly related, but does not indicate whether high intake is a cause or effect of high passage rate.

VIP stimulated total AA and individual AA intakes, but did not significantly increase AA supply to the duodenum. The duodenal protein consisted of over 70 % microbial AA estimated by DAPA. While previous reports indicated that VIP inhibits the absorption of leucine and alanine in rats (Chen et al., 1987; Nassar et al., 1995), VIP did not affect AA disappearance in the small intestine of sheep.

Evidence that VIP stimulates the secretion of prolactin, growth hormone and thyroid hormone (Zudenigo and Lackovic, 1989; Lam, 1991), and relaxes the ROO (Okine et al., 1995, 1996) show the potential of VIP to stimulate feeding in a complex way. Local infusion of VIP through a gastric artery catheter in the present study may have permited to act on local VIP receptors in the forestomach to reduce tension on the rumen wall and reduce activation of in-series tension receptors on the ruminal wall thus reducing signalling to satiety center. Also increasing the quiescence of the ROO to allow a large volume of flow, and inhibited

activity of the omasum to form a larger positive pressure difference between the reticulo-rumen and omasum may have helped to enhance intake. Although the short half-life of VIP may limit its action to the local regulation of gastrointestinal motility, digesta flow and intake behaviours, and VIP is cleared massively in the liver and lung, we can not rule out the possibility that VIP may have acted through a central neural path to influence feeding behaviour because there were relative changes in heart rate and pressure of the intestinal lumen by VIP (1-1.5 nmol min^{-1}) in the present study. Improving the secretion of other feeding stimulators such as prolactin by VIP needs to be explored.

As most previous reports, there were significant increases of feed intake and AA intakes in cold compared to warm environments (Westra and Christopherson, 1976; Kennedy, 1985; Sun et al., 1994), although contrary results are also reported (Chappel and Hudson; 1978; Sano et al., 1994). The variable responses to temperature may be due to different breeds, sex and fleece coat status in the different studies. The increased rate of passage of dietary residues from the ruminoreticulum to duodenum during cold exposure in the present study is in full agreement with previous reports (Westra and Christopherson, 1976; Kennedy, 1985). However, the present study is the first to examine in a comprehensive way these digestive responses in animals fed *ad libitum*. The significant interaction between VIP infusion and thermal environments indicated that VIP could sustain CP and AA duodenal passage in the warm, compared to that in the cold environment. It appears that VIP may reduce CP and AA degradation in rumen by the relaxation of the ROO to facilitate digesta outflow from the reticulo-rumen.

The data clearly indicates that VIP will increase voluntary feed intake in sheep when administrated intermittently over a 3 h period within an 8 h daily feeding schedule (i.e. when the VIP administration is spread over 40% of the available feeding time). When VIP was administrated over a 3 h period (i.e. 12%) of a 24 h feeding schedule, there was no significant effect on voluntary feed intake. This indicates that feeding stimulation by 30 min of total VIP infusion time is too short, compared to 24 h feeding time, to produce a significant effect. It is possible that administration of VIP throughout the full period of feed access might have enhanced voluntary intake. Further research is needed to answer this question.

VIP infusion did transiently depress EMG activities at the reticulum, ROO and omasum during periods of 3-8 min after commencement of VIP infusion in present study, but not when averaging the values over 10 min infusion periods. The transient effect of VIP observed here is similar to previous reports by Okine et al. (1995) and Reid et al. (1988b), except for shorter depression periods in the present studies. Okine and Mathison (1996) found that VIP decreased the frequency of reticular contraction compared to saline during the active feeding period, but this was not significant in the first infusion period in the present study if independently comparing the saline infusion. VIP effects were obviously overcome by the effect of stimulation associated with feeding.

Existence of significant differences in the frequency and quiescence of omasal EMG under different thermal environments in experiment three (Chapter one) indicates that the exposure to 1 ± 2 °C for closely shorn sheep was below the critical temperature. This is supported by the higher rates of heat production at 1 °C in the same experiment. The fleece growth during 3-7 weeks of acclimatization and trial periods was not sufficient to ameliorate the effects of the cold environment on myoelectric activities in sheep with body weights ranging from 40 to 60 kg.

Ingestion stimulated a large change in ROO diameter. Regular closure of the ROO happens at the first peak of reticular biphasic activity whereas the ROO opens widely after the ROO EMG peak or the second peak of reticular biphasic activity, at a time when the omasum is in relative relaxation or low activity. Irregular closure and opening of the ROO may also happen 0-2 times between each reticular contraction cycle, however, the incidence was lower than observations (3-5 min^{-1}) reported by Reid et al. (1988b) in anaesthetized lambs. Balch et al. (1951) reported that the lowest pressure in the omasum occurred 3 s after the second reticular contraction, coinciding with the opening of the ROO as noted in the present observation. Our results further confirm the endoscopic evidence for sheep by Ruckebusch (1970) that the ROO is closed when the reticulum is contracted. This closure may be due to the distortion of the musculature of the oesophageal groove, which surrounds this orifice, during contraction of the reticulum. However, the present data does not totally agree with the previous report that the ROO EMG activity corresponds to contractile closure

of the ROO (Reid et al., 1988a) because the initial time of ROO closure preceded the ROO EMG burst in the present study. Our data shows that the ROO is lightly open most of the time during the quiescence of the reticulum with one or more wider opening responses between cycle of reticular contractions. VIP infusion increasing duration of ROO opening after the second phasic contraction of reticular biphasic contractions may contribute to digesta outflow from the reticulo-rumen and ultimately improve intake. However, it is not clear how the wide opening of the ROO facilitates the digesta outflow. Therefore, the dynamic factors which regulate digesta outflow from reticulorumen to omasum require further study to be clarified.

VIP infusion significantly increased heat production (HP) in the cold environment during one experiment when the highest rate of heat production was recorde. However, it did not affect HP in the warm environment or average HP in both cold and warm environment. The reason for these differences in response is not clear. Under thermal exposures, the elevated HP and reduced rectal temperature in the cold, compared to warm exposure, indicated that there was an increased energy demand on the sheep, especially in experiment two.

VIP infusion caused a concentration-dependent increase of HR. Different infusion pathways have various effects on HR. Infusing VIP through the gastric artery at 1 nmol/min did not increase HR as much as infusion via the jugular vein. On the other hand, VIP at 3 nmol/min via the gastric artery catheter increased HR to the same level as VIP infused through the jugular vein. Clearance of VIP by the liver precludes a large increase in systemic concentrations when VIP is infused via gastric artery at low rate. Vice versa, VIP infusion through the jugular vein may also have its effect on gastrointestinal motility reduced because the lung is another major site of clearing VIP from the circulation. We have not attempted to verify these suggestions. However, The short effect of VIP on HR is similar to the observed phenomena which happened when investigating the effect of VIP on forestomach EMG activity. Maybe, the short duration of VIP effect could partially explain the lack of action on HP in most experiments, although VIP did stimulate a high heart rate in the present study.

B. General Conclusions

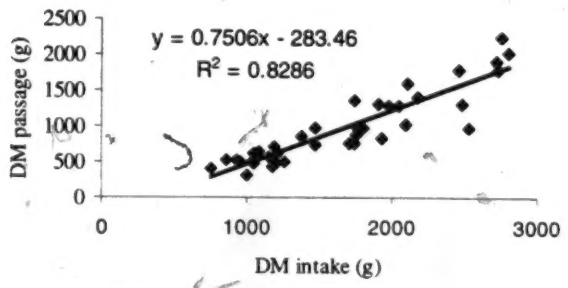
1. VIP stimulated intake when feed was available for 8 h per day, but not when feed was available for 24 h per day. VIP tended to increase the digesta flow from the ruminoreticulum to duodenum.
2. The increased feed intake when sheep are on an 8 h feeding schedule may have been due to the relaxation effect of VIP on ROO activity and increase of ROO opening.
3. Inhibition of VIP on gastrointestinal motility was transient.
4. VIP infusion significantly increased heart rate, however, the transient effect of VIP did not influence heat production and rectal temperature.
5. Cold exposure stimulated higher feed intake, gastrointestinal motor activity and increased nutrient supply to the intestine.

The results of this study suggest that VIP could contribute to variation in intake regulation in sheep. Under current conditions, VIP is not available in large quantities, is extremely expensive, and would definitely not be a cost-effective way to improve feed intake and nutrient supply in ruminants. However, this research has established the concept that VIP and VIP receptors play a significant role in regulation of feed intake in sheep. It may, therefore, be of value to examine potential technologies that could exploit this new knowledge. For example, there might be variation among animals in the expression of genes associated with VIP which influence voluntary feed intake, or there might be have some specific dietary components or agents capable of stimulating VIP release locally or rhythmically. Learning how VIP is normally released in the reticulorumen could represent

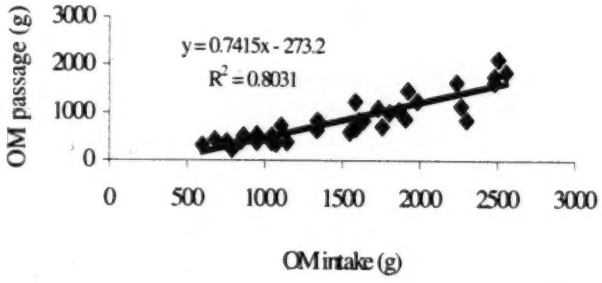
an interesting focus for future research, as substantial individual animal variation is known to exist for voluntary feed consumption.

Figure 7-1. Regression of dry matter (DM), organic matter (OM) and amino acid (AA) passage at the duodenum on feed intake of DM (A), OM (B) and AA (C).

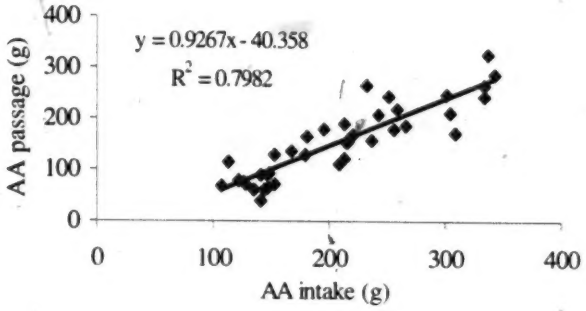
A.



B



C



C. Literature Cited

Balch, C. C., A. Kelly, and G. Heim. 1951. Factors affecting the utilization of food by dairy cows. 4. The action of the reticulo-omasal orifice. Br. J. Nutr. 5: 207-216.

Bueno, L. 1972. Action du sphincter reticulo-omasal chez les bovins. Annls. Rech. Vétér. 3: 83-91.

Chappel, R. W., and R. J. Hudson. 1978. Winter bioenergetics of Rocky Mountain bighorn sheep. Canadian Journal of Zoology. 56 (11): 2388-2393.

Chen, Y. F., Z. T. Feng, S. H. Wen, and G. J. Liu. 1987. Effect of vasoactive intestinal polypeptide, somatostatin, neurotensin, cholecystokinin octapeptide, and secretin on intestinal absorption of amino acid in rat. Digestive Diseases & Sciences. 32: 1125-1129.

Harmon, D. L., and C. J. Richards. 1997. Considerations for gastrointestinal calculations in ruminants. J. Anim. Sci. 75: 2248-2255.

Kennedy, P. M. 1985. Influences of cold exposure on digestion of organic matter, rates of passage of digesta in the gastrointestinal tract, and feeding and rumination behaviour in sheep given four forage diets in the chopped, or ground and pelleted form. British Journal of Nutrition. 53(1): 159-173.

Lam, K. S. 1991. Vasoactive intestinal peptide in the hypothalamus and pituitary. [Review] Neuroendocrinology. 53 (Suppl 1): 45-51.

Nassar, C. F., L. E. Abdallah, K. A. Barada, S. F. Atweh, and N. E. Saade. 1995. Effects of intravenous vasoactive intestinal peptide injection on jejunal alanine absorption and gastric acid secretion in rats. Regulatory Peptides. 55(3): 261-7.

Okine, E. K., and G. W. Mathison. 1996. Effects of vasoactive intestinal polypeptide on electromyographic activity of the reticulo-omasal orifice in sheep at two levels of intake. Journal of Animal Science. 74(7): 1681-8.

Okine, E. K., J. O. O. Miaron, S. Cosgrove, and R. J. Christopherson. 1995. Vasoactive intestinal polypeptide inhibits electromyographic activity of the reticulo-omasal orifice in meal-fed sheep in warm and cold environment. Can. J. Anim. Sci. 75:333.

Reid, A. M., A. Shulkes, and D. A. Titchen. 1988a. Effects of the vagus nerves on gastric

mobility and release of vasoactive intestinal peptide in the anaethetized lamb. J. Physiol. 396:11--24.

Reid, A. M., A. Shulkes, and D. A. Titchen. 1988b. The vasoactive intestinal polypeptides on gastric motility in the lamb. J. Physiol. 396:41--45.

Ruckebusch, Y. 1970. The electrical activity of the digestive tract of the sheep as an indication of the mechanical events in various regions. J. Physiol. 210: 857-882.

Ruckebusch, Y. 1988. Motility of the gastro-intestinal tract. In: Church, D.C., ed. The Ruminant Animal. Englewood Cliffs, NJ.

Sano, H, H. Takaya, N. Hasemi, and Y. Terashima. 1994. Effects of cold exposure and intravenous glucose infusion on feed intake and plasma insulin response to feeding in sheep. Animal Science and Technology. 65(1): 1-8

Sun, Y. X., J. Lee, P. M. Harris, E. R. Sinclair, I. D. Shelton, H. T. Blair, and S. N. McCutcheon. 1994. Nitrogen and sulfur metabolism and plasma thyroid hormone concentrations in fleeceweight-selected and control Romney sheep at two ambient temperatures. Australian Journal of Agricultural Research. 45(2): 339-354.

Westra, R., and R. J. Christopherson. 1976. Effects of cold on digestibility, retention time of digesta, reticulum motility and thyroid hormone in sheep. Can. J. Anim. Sci. 56:699--708.

Zudenigo, D., and Z. Lackovic. 1989. Vasoactive intestinal polypeptide: a potential neurotransmitter. Lijecnicki Vjesnik. 111(9-10): 354-9.